S. F. W. Sa
#60

#60

LIMNOLOGICAL ORGANIC ANALYSES BY QUANTITATIVE DICHROMATE OXIDATION

LIBRARY
FEB 20 1963
FISH & WILDLIFE SERVICE

UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE
BUREAU OF SPORT FISHERIES AND WILDLIFE
RESEARCH REPORT 60

RESEARCH REPORTS

This series of reports was begun when the Fish and Wildlife Service was established in 1941; the series superseded Wildlife Research Bulletins of the Bureau of Biological Survey and Investigational Reports of the Bureau of Fisheries, those two bureaus having merged to form the Service. The Research Reports are being continued (with Research Report 54) in a slightly changed format, as the research-reporting series of the Bureau of Sport Fisheries and Wildlife (U.S. Fish and Wildlife Service), comprising reports of scientific investigations related to the work of the Bureau. The Bureau distributes these reports without charge to official agencies, to libraries, and to researchers in fields related to the Bureau's work; additional copies may usually be purchased from the Division of Public Documents, U.S. Government Printing Office.

Current Research Reports are—

54. Fluctuations in Age Composition and Growth Rate of Cutthroat Trout in Yellowstone Lake, by Ross V. Buckley. 1961. 31 pp.
55. Mortality Studies on Cutthroat Trout in Yellowstone Lake, by Orville P. Ball and Oliver B. Cope. 1961. 62 pp.
56. Limnology of Yellowstone Lake in Relation to the Cutthroat Trout, by Norman G. Benson. 1961. 33 pp.
57. Chemical Composition of Blood of Smallmouth Bass, by Eddie Wayne Shell. 1961. 36 pp.
58. Chronic Effects of Endrin on Bluntnose Minnows and Guppies, by Donald Irvin Mount. 1962. 38 pp.
59. Predicting Year-Class Abundance of Yellowstone Lake Cutthroat Trout, by Ross V. Bulkley and Norman G. Benson. 1962. 21 pp.
60. Limnological Organic Analyses by Quantitative Dichromate Oxidation, by John A. Maciolek. 1962. 61 pp.
61. Bioassay and Use of Pituitary Materials to Spawn Warm-Water Fishes, by Howard P. Clemens and Kermit E. Sneed. 1962. 30 pp.
62. Equilibrium Yield and Management of Cutthroat Trout in Yellowstone Lake, by Norman G. Benson and Ross V. Bulkley. In press.

LIMNOLOGICAL ORGANIC ANALYSES BY QUANTITATIVE DICHROMATE OXIDATION

John A. Maciolek
Fishery Research Biologist

RESEARCH REPORT 60

UNITED STATES DEPARTMENT OF THE INTERIOR
Stewart L. Udall, *Secretary*

Frank P. Briggs, *Assistant Secretary*
for Fish and Wildlife

FISH AND WILDLIFE SERVICE
Clarence F. Pautzke, *Commissioner*

BUREAU OF SPORT FISHERIES AND WILDLIFE
Daniel H. Janzen, *Director*

Published by the Bureau of Sport Fisheries and Wildlife • Washington • 1962
Printed at the U.S. Government Printing Office, Washington, D.C.

For sale by the Superintendent of Documents, U.S. Government Printing Office
Washington 25, D.C. - Price 40 cents

CONTENTS

		Page
I.	Introduction	1
II.	Oxidants and oxidative procedures	2
	Permanganate and other oxidants	3
	Dichromate	4
	A general procedure for dichromate oxidation	5
III.	Reaction characteristics of dichromate and organic matter	6
	Properties of dichromate	6
	Organic compounds as reductants	7
	Oxidation of naturally occurring organic matter	9
	Factors affecting the efficiency of dichromate oxidation	10
	The oxidation potential: reagent concentration and temperature	10
	Nature of the organic substrate	12
	Reaction time and catalysis	13
	Interferences	15
IV.	Methods of dichromate oxidative analysis	17
	Preanalytical sample treatment	17
	Quantitative oxidations	20
	Semimicro method	20
	Micro method	22
	Discussion	24
	Determination of organic elements	26
	Carbon analysis	27
	Nitrogen analysis	30
	Concluding remarks	33
V.	Significance and utilization of analytical data	33
	Transformations of Oxygen Consumed values	33
	Direct estimation of total organic matter	34
	Estimation of organic carbon	36
	Estimation of proximate composition	38
	Estimation of organic energy	38
	Utilization of Oxygen Equivalent values	40
	Determination of proximate composition	40
	Determination of ultimate composition	42
	Concluding remarks	43
VI.	Limnological examples of quantitative oxidation	43
	Total organic content of alpine lakes	44
	Quantitative periphyton analysis	45
	Plankton and neuston composition	48
VII.	Summary	49
Bibliography		51
Appendix—Proximate components in limnological samples		57

ABSTRACT

A limnological oxidation technique, based on procedures employed in soil chemistry, was developed and tested by use of purified organic compounds and various natural substrates. A sample is oxidized by dichromate in a strongly acid medium (2 H_2SO_4 to 1 H_2O, by volume) maintained at 100° C. The amount of dichromate reacting (determined titrimetrically as the difference between the initial and final quantities) is reckoned as Oxygen Consumed, or weight of oxygen required by the sample. The procedure is adapted to samples of different sizes by adjusting the volume or normality of the dichromate. Under optimal conditions, resistant compounds are oxidized with an efficiency of 95 percent or more. The oxidation also serves as the principal step for sensitive determinations of organic carbon (as CO_2) and nitrogen (as NH_3). Both elements are estimated by titration after diffusion separation—the carbon absorbed in hydroxide concurrent with oxidation and the nitrogen diffused subsequently from the reacted mixture into a boric acid solution. These analyses require no special apparatus or complicated procedures. They are sensitive to as little as 0.01 milligram of organic matter, precise to within $\pm$ 1 percent in the semimicro range, and adaptable to a variety of sample materials and to multiple simultaneous determinations.

LIMNOLOGICAL ORGANIC ANALYSES BY QUANTITATIVE DICHROMATE OXIDATION

By John A. Maciolek, *Fishery Research Biologist*
Bureau of Sport Fisheries and Wildlife

Part I. INTRODUCTION

Estimation of the quantity of organic matter in aquatic samples is difficult because of its different physical states and its low concentration relative to mineral matter and water. Probably the most common primary measurement of total organic matter is a mass estimate made by determining the loss in weight upon ignition of a sample that has been evaporated to dryness. Ignition loss, being an indirect measurement, carries an error proportional to the amount of inorganic matter present, and this error is usually too large for meaningful interpretation of analytical results.

In some circumstances, the determination of energy rather than mass is a more important primary measurement of sample organic matter (cf. MacFadyen, 1949). The direct estimate of organic energy, as done by bomb calorimetry, is difficult to adapt to limnological samples. This technique also has the disadvantages of being analytically exacting and of not allowing rapid or multiple simultaneous determinations.

Various other measurements provide information which more fully describes the organic nature of a sample. Aquatic organic matter has been characterized in this manner by specific molecular or elemental constituents, and by its proximate composition. These determinations involve techniques such as chromatography, spectrophotometry, combustion analysis, and solvent extraction. Limnological adaptions of standard analytical methods may be criticized as having insufficient sensitivity or accuracy, or as being too complicated for the skills and facilities of the average researcher. Dissolved organic matter, especially, has been inadequately studied for lack of a simple, direct method of analysis. The key problem in investigating aquatic organic matter, then, is methodological.

Need for a better method of measuring total organic matter arose particularly in connection with continuing investigations of strongly oligotrophic lakes in the Sierra Nevada of California. Net plankton was not detected in some of these lakes (Reimers et al., 1955) and in no case did ignition loss exceed 6 milligrams per liter. At the lower extreme, the concentration of total dissolved solids in a small cirque lake (Reimers, 1958) was about 8 milligrams per liter, and the ignition loss was only 0.2 milligrams per liter. The situation required an analytical method that was simple, sensitive to low levels of organic matter, and preferably adaptable to multiple simultaneous determinations. The original objective in developing such a method was to permit within-lake and between-lakes comparisons of total organic matter in terms of mass or energy. The principle of quantitative

oxidation, or decomposition of a sample with a measured amount of oxygen, appeared to be a suitable basis upon which to develop the desired method.

Quantitative oxidation analysis is based on the reduced state of organic matter. Consider a "typical" molecule as metabolically constructed from CO_2, H_2O, and NH_3 by removal of some of the oxygen atoms and condensation of the remainder into a unit having a framework of carbon with all of the atoms covalently bonded. The ratio of these atoms with respect to their valences must establish electronic neutrality within the molecule. The formation process is one of reduction and requires energy in proportion to the degree of reduction. This process is reversed in chemical oxidation to yield the original inorganic constituents. The amount of oxygen required is proportional to the energy content and is also an indirect estimate of the quantity of organic matter oxidized.

Quantitative oxidations are most conveniently accomplished with combined oxygen, that is, in relatively stable reagents which can be reduced by organic matter under certain reaction conditions. A known excess of oxidant is provided, and the remainder is determined after the reaction has been completed. The amount of reacting oxygen is the equivalent difference between the two values. These reactions, whether quantitative or not, are commonly referred to as "wet oxidations."

Appraisal of existing methods indicated that the oxidative reaction could also serve as an initial step in procedures for analyzing component elements. The problem was therefore expanded to include the development of simple accessory analyses for carbon and nitrogen as well as the necessary interpretation of all empirical results in terms of natural organic matter. This analytical scheme is propounded here as an approach to the general characterization of organic matter in all types of limnological samples.

Part II. OXIDANTS AND OXIDATIVE PROCEDURES

An extensive survey was made of literature concerning the analysis of organic matter by oxidative methods in order to determine the most suitable reagents and procedures for quantitative limnological oxidations. Primary emphasis was placed on hydrochemical techniques and on reports concerning quantitative oxidation with dichromate, and the survey was intensive in this respect.

There are many reagents which can serve as organic oxidants, but these cannot be compared solely on a theoretical basis since their characteristics will vary under different reaction conditions; comparisons must be made with respect to the procedures in which they are used. In terms of the objectives of this study, the desirable characteristics of a procedure for quantitative oxidation are—

1. Efficiency and accuracy; organic matter should be completely oxidized as rapidly as possible with the least interference from inorganic constituents.

2. Sensitivity and precision; differences between samples should be distinguishable at a microanalytical level with a minimum of replication.

3. Simplicity and versatility; the procedure should require a minimum of essential equipment and operations as well as numbers and amounts of reagents, and should apply equally well to dissolved or particulate matter over a wide range of sample sizes, regardless of the amount of inorganic matter present.

PERMANGANATE AND OTHER OXIDANTS

Potassium permanganate has been employed as a quantitative oxidant in hydrochemistry for more than a century. A comprehensive review of its history, methodology, and effectiveness in organic oxidation was presented by Meissner (1958). Meissner attributed the introduction of permanganate methods of water analyses to Forschammer in 1849, when Forschammer was attempting to overcome the limitations of ignition loss gravimetry. According to Mason (1890), Forschammer's original technique merely required the addition of standard permanganate solution to a water sample until its color persisted. Subsequent improvements consisted in adding acid or hydroxide to the sample, and heating the mixture to increase the reaction rate (Kubel technique). Meissner (1958) stated that none of the many method variations proposed significantly improves upon the 70-year-old Kubel technique. More recent literature dealing with limnological methods and applications of permanganate oxidation includes Åberg and Rodhe (1942), Berg and Petersen (1956), Braun (1952), Dobrzhanskaya (1956), Elster (1959), Gillbricht (1957), Juday and Birge (1932), Matsue (1950), Maucha (1932), Ohle (1940), and Wereščagin et al. (1932). Generally, oceanographic investigators have found that permanganate methods are inapplicable to seawater analyses (Lopez-Benito, 1955; Duursma, 1960).

Permanganate has several disadvantages as an analytical reagent (Kolthoff and Sandell, 1952), the worst being its tendency toward autodecomposition. Because of this instability, it cannot be employed in the rigorous reaction conditions necessary for complete oxidation of organic matter. Despite the many methods proposed, permanganate usually oxidizes less than half of the total organic matter present. Juday and Birge (1932) concluded that the permanganate method they used was about 40 percent effective in oxidizing organic carbon. Permanganate and dichromate oxidations were used by Votintsev (1953, 1955) in analyzing Lake Baikal samples, from surface foam to a depth of 1,200 meters. His data show that the oxidation values obtained with permanganate were only 10 to 54 percent of those yielded by dichromate.

The oxidative effectiveness of permanganate in both acid and alkaline reaction was compared with that of acid dichromate using the procedure described in part IV. Samples of filtered hatchery water, analyzed by the three methods, gave the results in table 1. Since the dichromate oxidation was approximately 90 percent complete, permanganate oxidized only about 30 percent of the organic matter present.

Low and erratic results of permanganate oxidations with natural organic matter can be better understood from the data of Meissner (1958), which concern the permanganate oxidizability of more than 200 organic compounds. Not only are the recoveries generally low, but there is no consistency of results within the various groups of compounds (amino acids, carbohydrates, fatty acids).

TABLE 1.—*Results from permanganate and dichromate oxidations of dissolved organic matter in trout-hatchery water at Cortland, N.Y.*

Sample analyzed	Oxygen consumed, milligrams per liter [1]		
	Acid permanganate	Alkaline permanganate	Acid dichromate
Influent (supply stream)	0.63	0.10	1.86
Effluent (from raceways)	1.51	1.06	4.53

[1] All values are unadjusted means of duplicate determinations. The acid permanganate method was that used by Juday and Birge (1932), the alkaline permanganate method is from Maucha (1932), and the dichromate oxidation is described in this report under "Micro method."

Certain other oxidants and methods of oxidation are worthy of note in that they pertain either to water analysis or to the treatment given organic matter in this study. Medalia (1951) has suggested an interesting and sensitive partial oxidation for detecting traces of organic matter in water. It utilizes ferrous iron and hydrogen peroxide as reagents, and these react with certain organic linkages in a chain reaction which oxidizes both the iron and the organic linkage. More recently, Slater (1954) described a technique for quantitatively oxidizing organic matter in natural waters with ammonium nitratocerate and perchloric acid. Ceric sulfate, periodate, and other oxidants have been used effectively in quantitative organic oxidations (Buydens and Ledent, 1957; Skopintsev, 1949c, 1950; Urbanowiczówna, 1947).

Dry combustion methods may also serve as quantitative oxidations, but the procedures are generally more difficult. Krogh (1930) employed a cupric-oxide combustion on dried organic residues, with gasometry to measure the end products and the oxygen needed to reoxidize the copper. The proximate composition and energy content of the sample were calculated from these values. Krogh has applied this method limnologically (Krogh and Berg, 1931; Krogh and Lange, 1932). Ultimate analysis by a standard dry combustion-absorption train enabled Spoehr and Milner (1949) to calculate indirectly the oxygen required for combustion of algal samples. This oxygen was expressed as the "R-value" and was used to determine the proximate composition of the samples.

DICHROMATE

Chromic acid and its salts have been used effectively in oxidizing organic matter by procedures developed in a number of investigational fields. Potassium dichromate, in particular, appears to be the most suitable oxidant for the present problem and offers the following advantages:

It is a primary standard.

It is very effective in oxidizing most organic compounds under suitable reaction conditions.

It is extremely stable in neutral solution, and also in acid solution if protected from contamination.

It does not decompose spontaneously to any appreciable extent during a reaction, even at high temperatures and acid concentration.

It can be measured colorimetrically or titrated by several common reductants to a very sharp endpoint.

It can be used in procedures which require no unusual precautions.

It contains (or requires for reaction) no elements which are known to interfere with the accessory analyses considered later.

One of the earliest applications of dichromate in organic oxidations was reported by Rogers and Rogers (1846, 1848) who found it superior to "peroxide of manganese" in preparative reactions for formic and acetic acids. Subsequently, many dichromate techniques were developed for biochemical analyses, such as the quantitative oxidations described by Christensen et al. (1937), Ivlev (1934), Johnson (1949), and Kuhn and L'Orsa (1931).

Various hydrochemical analyses employing dichromate have appeared in recent years. Direct carbon determinations by nonquantitative oxidation was described by Forsblad (1955), Kay (1954), Krogh and Keys (1934), Krylova (1953), Mohlman and Edwards (1931), and Skopintsev (1949a). Quantitative oxidations have been developed for the sanitation-pollution field (A.P.H.A., 1955; Moore et al., 1949; Moore and Walker, 1956). In limnology, quantitative dichromate analyses have been developed primarily in Russia. Vin-

berg et al. (1934) designed a simple technique and employed it on a unique study of the benthos of Lake Beloje. Vinberg's technique has been modified and adapted for the analysis of dissolved organic matter, one of the more recently described methods being that of Nikolaeva (1953). The studies of Votintsev (1953, 1955) on Lake Baikal provide an example of the application of dichromate oxidation to the measurement of dissolved organic matter. Another simple dichromate technique for estimating organic carbon in water samples was given by Šesták (1958). Procedures for marine analyses were described by El Wakeel and Riley (1957) and Strickland and Parsons (1960).

Analytical problems similar to those of limnology are encountered in the field of soil chemistry where the history of oxidative analysis for total organic matter and organic carbon has been traced by Clark and Ogg (1942). Dichromate was first employed as a combustion reagent in the direct determination of organic carbon (Ames and Gaither, 1914). Modifications of this procedure are still being proposed (Shaw, 1959; Allison, 1960). The use of a quantitative oxidation to determine soil organic content is generally attributed to Schollenberger. His "rapid approximate" method (Schollenberger, 1927) has been variously modified (Allison, 1935; Graham, 1948; Schollenberger, 1945; Tiurin, 1931; Walkley and Black, 1934). Perhaps the simplest and most widely used technique of this type is the one described by Peech et al. (1947).

A GENERAL PROCEDURE FOR DICHROMATE OXIDATION

Methods described in the literature were evaluated to select the best procedure upon which to develop the desired analytical techniques. Oxidation of a sample is commonly accomplished with a large excess of dichromate and a high concentration of sulfuric acid. Heat is employed to hasten the reaction, and the excess dichromate is determined titrimetrically after diluting the reactants. Procedures differ in several minor respects that make them more or less acceptable. Some objectionable features noted were inherently poor precision or accuracy, the need for special apparatus, and the lack of sensitivity or adaptability to samples of various types and sizes. "Rapid" methods used in soil analysis seemed most desirable because of their simplicity, versatility, and potential usefulness in limnology.

The following is a general outline of the oxidation procedure which is of concern in the remaining text. It is based on the Walkley method (Peech et al., 1947), initially modified by extending the reaction time in a boiling water bath to enable more effective oxidation of resistant compounds.

1. A sample (moist, dry, or dispersed in a small quantity of water) is placed in a suitable heatproof flask.

2. A known amount of potassium dichromate is added to the sample in excess of that required for complete oxidation.

3. Concentrated sulfuric acid is added in an amount equal to twice the volume of water present, and the flask is swirled.

4. The flask is lightly covered and placed in a boiling water bath until the reaction is considered complete.

5. The reaction mixture is diluted and cooled, and its excess oxidant is titrated (ferrous solution). Oxygen consumed is derived from the difference between sample and reagent blank titers.

Part III. REACTION CHARACTERISTICS OF DICHROMATE AND ORGANIC MATTER

All organic compounds are susceptible to oxidation by dichromate under certain conditions. These conditions impose restrictions on the design of a suitable quantitative procedure. Because the reaction is neither simple nor instantaneous, consideration must be given to the properties of the reactants and to the factors which affect their reactive efficiency. The analysis must be expressed quantitatively according to the relation between organic matter and oxygen, reacting as dichromate. Two expressions (Oxygen Equivalent and Oxygen Consumed) are proposed to satisfy both theoretical and empirical oxygen-organic matter relations.

PROPERTIES OF DICHROMATE

Dichromate ions ($Cr_2O_7^{-2}$) form orange-colored solutions which reduce to green or bluish-violet chromic (Cr^{+3}) derivatives. They are strong oxidizing agents in the presence of free acids. The proton requirement in this reaction is given by the ionic equation

$Cr_2O_7^{-2} + 14H^+ + 6e \rightarrow 2Cr^{+3} + 7H_2O$,

which is generalized because ions of intermediate valence occur in the reduction of chromate to chromic chromium. Reagent-grade potassium dichromate (molecular weight = 294.21) is a primary standard; its equivalent weight, indicated in the reaction above, is 294.21/6 or 49.04. The standard oxidation potential, E_o (at 25° C. with reference to *N* hydrogen electrode, pH = 0), of this reaction is about − 1.35 volts. The actual oxidation potential of any dichromate system varies with temperature, pH, and dichromate-chromate ion concentrations according to the relations

$$E = E_o + \frac{0.0001983\ T}{6} \log \frac{(H^+)^{14}\ (Cr_2O_7^{-2})}{(Cr^{+3})^2}$$

where E = observed potential, E_o = standard potential, and T = absolute temperature.

Dichromate is essentially an acid salt. It reacts with hydroxyl ions to form yellow chromates (CrO_4^{-2}) and water. Chromic ions (from reduced dichromate) also react with hydroxyl ions to produce green chromites ($Cr(OH)_4^{-1}$). These alkaline reactions do not involve electron changes and therefore are not oxidations. They will be referred to later in connection with neutralizing the reaction mixture.

The stoichiometric relations involved in the titration of dichromate with ferrous sulfate are expressed by the equation $6FeSO_4 + K_2Cr_2O_7 + 7H_2SO_4 \rightarrow K_2SO_4 + 3Fe_2(SO_4)_3 + Cr_2(SO_4)_3 + 7H_2O$. The relation between oxygen and a reductant in an oxidative reaction is indicated also. Six equivalents of oxygen, as dichromate, are required for the complete oxidation of 6 moles (or 6 equivalents) of ferrous sulfate. Since the equivalent weight of oxygen is 8, and that of ferrous sulfate is 151.92, 8/151.92 or 0.0527 represents the weight of oxygen required per unit weight of ferrous sulfate. This ratio (eq. wt. oxygen/eq. wt. reductant), with equivalents expressed in the same units, is referred to hereafter as the Oxygen Equivalent (O.E.) of a reductant. Oxidizable compounds or mixtures of such compounds are distinguishable by their characteristic O.E.'s. Oxygen Equivalents can be determined experimentally as well as mathematically. The weight of oxygen used in a reaction, divided by the weight of the substrate reduced (pure compound or mixture), is the fundamental relation of analysis by quantitative oxidation.

ORGANIC COMPOUNDS AS REDUCTANTS

Organic matter has been described earlier as carbon in a state of reduction, the degree of this reduction dependent upon the character and quantity of other elements present. Oxidation returns these substituents to their original states. Consider a compound composed of the four primary organic elements: carbon, hydrogen, oxygen, and nitrogen. The products of complete reaction of this compound with dichromate in the procedure used here are CO_2, H_2O, and NH_3. Representing this compound with the amino acid glycine, the simple oxidative reaction would be

$$(1)\ C_2H_5O_2N + 1\tfrac{1}{2}O_2 \rightarrow 2CO_2 + H_2O + NH_3.$$

The ammonia forms a salt in acid solution. Reaction kinetics of organic matter are not well understood, but the following balanced equation illustrates its general reaction with dichromate:

$$(2)\ 2K_2Cr_2O_7 + 2C_2H_5O_2N + 9H_2SO_4 \rightarrow 4CO_2 + 10H_2O + (NH_4)_2SO_4 + 2Cr_2(SO_4)_3 + 2K_2SO_4.$$

Equation 2 serves only to demonstrate the stoichiometric relations. It is otherwise unnecessary to write a balanced equation for the dichromate reaction, and this operation becomes more difficult as molecular size increases. The relation of the reductant to oxygen is more easily seen in equation 1. One mole of glycine requires 1.5 moles of oxygen, and its Oxygen Equivalent in gram ratio is 48/75.1 or 0.639 grams of oxygen per gram of glycine.

The Oxygen Equivalent of any organic compound is most easily calculated by a simple valence-change method. Since oxidation-reduction is an electron-transfer process, the net change in valence which a reductant undergoes in a reaction indicates the amount of oxygen required (1 electron = 1 equivalent of oxygen). It is necessary to know, or to correctly assume, the valences of the elements before and after they have reacted. In the case of most inorganic reducing agents, the valences of the ions are obvious because of molecular polarity. Organic molecules, however, are bonded mainly by shared electrons (covalent bonds), and the assignment of valence to the elements becomes problematical. A practical method assigns the shared electrons to elements other than carbon according to the valence properties they display in normal inorganic states. Thus, oxygen is ordinarily negative and hydrogen is positive, and they are given the valences -2 and $+1$, respectively. Organic nitrogen, if present, is considered negative (amino, amide, etc.) and to it is ascribed a -3 valence. The effective valence of carbon in the molecule must therefore be a value which establishes electrical neutrality, or the sum of the valences of the other elements but opposite in sign. For glycine, the valence distribution is

$H_5 = +5$, $O_2 = -4$, $N = -3$, which total -2, and thus $C_2 = +2$.

This manner of assigning valence simplifies the interpretation of relations among the reaction products, for it is evident from equation 1 that the net valence change in the reaction is attributable to carbon alone. The total valence of carbon in the two moles of CO_2 produced is $+8$ (2 CO_2 contain 4 oxygens with a total valence of -8). The net valence change in the reaction is $+2 \rightarrow +8$, or 6 electrons per mole of glycine. The O.E. of glycine is $6 \times 8/75.1$, or 0.639 grams of oxygen per gram of glycine.

Calculation of the Oxygen Equivalent for a compound of known formula can be summarized in four steps:

1. Determine the effective valence of carbon in the compound by multiplying the number of atoms of each other ele-

ment by its assigned valence (H = +1, O = −2, N = −3) and summing these values with regard to sign. The carbon valence is the difference between the sum and zero.

2. Determine the valence of carbon as CO_2 by multiplying the number of C atoms by 4 (it will have a positive sign).

3. The difference between the valences of carbon in Steps 1 and 2 is the net valence or electron change of the reaction. It is equal to the equivalents of oxygen required (multiply by 8 to obtain the weight of oxygen).

4. The weight of oxygen divided by the molecular weight of the organic compound is its Oxygen Equivalent.

This method of calculating an O.E. applies to natural organic compounds wherein nitrogen carries a negative valence. Compounds containing nitrogen in a positive valence state require a revised valence assignment. Some compounds may contain elements other than those mentioned. Chloride is oxidized to free chlorine with a valence change of 1 electron. Other halides react similarly. Reduced sulfur is oxidized to SO_3 (or SO_4^{-2}). The maximum valence change for sulfur is +8, represented by the oxidation of sulfhydryl S (as in cysteine) to sulfate. It is necessary to add such valence changes to the net valence change of carbon when calculating the O.E. of a compound which contains these oxidizable elements. Some carbohydrate materials, such as agars and mucins, contain oxidized sulfur (as sulfamic groups or -OSO_3- linkages) which requires no oxygen in reaction.

An Oxygen Equivalent may be calculated for a mixture of 2 or more compounds whose proportionate quantities are known by taking a weighted mean of their individual O.E.'s. It is also possible to determine the O.E. of organic matter from its ultimate composition by the method described above. The elemental percentages first must be converted into atomic ratios as they would appear in a hypothetical molecule. Assume that a sample of organic matter has the same ultimate composition as glycine: 32.0 percent C, 6.7 percent H, 42.7 percent O, 18.7 percent N. These percentages are divided by their respective atomic weights to give a hypothetical molecule whose weight is 100: $C_{2.66}H_{6.66}O_{2.66}N_{1.33}$. The valence change is found as outlined above:

$$\begin{aligned} H_{6.66} &= +6.66 \\ O_{2.66} &= -5.32 \\ N_{1.33} &= -3.99 \\ \hline C_{2.66} &= +2.65 \rightarrow 2.66C \text{ (as } CO_2) \\ &= +10.64 \end{aligned}$$

The net valence change is 10.64−2.65 = 7.99, and the weight of oxygen required is 7.99 × 8, or 63.92. The O.E. of the sample material is, then, 63.92/100 = 0.639. This is a convenient way to determine the O.E. of a sample of unknown molecular composition, but whose elemental analysis is available. It presumes that none of the elements is present in inorganic combination.

There are two important assumptions concerning end products in the foregoing treatment which require a final qualification. The first is that all reduced nitrogen goes unoxidized and appears eventually as ammonium salts. There are no definite indications of nitrogen oxidation in the procedures employed here, which agrees with the observations of Williams (1937). However, Ark (1928) stated that imino nitrogen is oxidized by a sulfuric acid–dichromate mixture. The extent to which this might occur is not known. Nonoxidation of organic nitrogen does not hold true if prosphoric acid is included in the reaction mixture as suggested by Walkley (1947) and used by Allison (1960), Lawton (1955), and Mohlman and Edwards (1931). Christensen et al. (1937) found that the inclusion of phosphoric acid caused a nearly complete conversion

of NH_3 to nitrate. The possible oxidation of some forms of organic nitrogen by the present dichromate procedure remains to be demonstrated. Should this occur, however, the overall effect on oxidative considerations of natural organic matter would be slight.

The second assumption to be qualified concerns incomplete oxidation which probably occurs to a limited extent in all reactions. Schollenberger (1945) indicated that some carbon monoxide may escape oxidation, and this phenomenon has been observed also by Christensen et al. (1937). However, it has not been noted in this study or by many other workers employing dichromate, even in "weak" partial oxidations. Since CO is quite readily oxidized, a thorough and prolonged mixing of the reactants as they are combined should preclude its evolution. Formic acid is a product of weak oxidation but apparently does not escape decomposition under strong oxidation potentials (Ark, 1928; Kuhn and L'Orsa, 1931). Other low molecular weight acids may be volatilized from a reaction mixture before they are decomposed. The only one to do this to an appreciable extent is acetic acid, which is quite resistant to oxidation and apparently is formed from ethoxyl or potential ethoxyl groups during the reaction (Kuhn and L'Orsa, 1931). The complete oxidation of acetic acid and other volatile unoxidized products is encouraged by catalysts and strong oxidation potentials. The effects of these factors will be considered later.

OXIDATION OF NATURALLY OCCURRING ORGANIC MATTER

The purpose of quantitative oxidation is to characterize organic matter from natural samples by its relation, in reaction, to oxygen. This fundamental relation is termed here, the Oxygen Equivalent. It is possible, in situations where the quantity of organic matter in a sample is known, to estimate an O.E. experimentally. More commonly, a sample is oxidized, and the resulting value is taken to represent, directly or by conversion, the amount of organic matter it contains. This oxidation parameter requires a term to distinguish it from the O.E., which is a distinct chemical property. It has been referred to in the literature in various ways: Chemical Oxygen Demand (C.O.D.), Oxygen Consumed, Oxygen Consuming Capacity, Oxidizability, Oxygen Demand, Oxygen Required, and Reducing Power. There is further confusion, especially in abbreviation, with expressions that refer to different parameters: Biochemical Oxygen Demand (B.O.D.), Dissolved Oxygen (D.O.), Oxygen Deficit, and the compound adjective, Oxidation-Reduction (O–R). To eliminate duplicate terminology and the possibility of confusion, the term Oxygen Consumed (O.C.) is proposed exclusively for the parameter in question.

These two oxidation terms can be defined, with special reference to natural organic matter, as follows:

The Oxygen Equivalent (O.E.) is the weight of oxygen required for complete oxidation of a unit weight of organic matter.

The Oxygen Consumed (O.C.) is the weight of oxygen required in the oxidation of any sample containing organic matter.

The O.E. is always a ratio, while the O.C. is simply a quantity of oxygen, as stated above. It is frequently desirable to express Oxygen Consumed as a ratio, that is, as the weight of oxygen required per unit weight, volume, or surface area of sample. The difference between O.C. and O.E. as weight ratios is in the distinction between true "organic matter" and "sample" (partly organic). These two values (as weight ratios) will be equal when the sample is purely organic.

Spoehr and Milner (1949) have used the expression "R-value," or "degree of reduction," in the oxidative characterization of algal matter. Briefly, it is definable as the quantity of oxygen required for the combustion of a unit weight of organic substrate, calculated on a 0–100 scale. The larger figure represents a maximum value for the most reduced compound (methane). The R-value concept includes the oxidation of organic N to N_2, but in other respects it is similar to the Oxygen Equivalent (for nonnitrogenous compounds, R-value $\times$ 0.04 = O.E.). The R-value does not refer to oxygen by weight directly (because it is a double ratio) and therefore lacks the utility of the O.E. The 0–100 scale, moreover, has little advantage in classifying organic matter over the comparable 0–4 O.E. scale.

In connection with the analysis of total organic matter in natural samples, it should be noted that the total organic substance, and not the carbon alone, is oxidized. Although the theoretical Oxygen Equivalent has been defined here in terms of carbon valence change, the initial carbon valence is ascribed to the other elements present, and thus they are directly involved. Juday and Birge (1932), in estimating the efficiency of their oxidative analyses in terms of carbon, stated that 2 atoms of oxygen (or 32 g.) are required per atom of carbon (12 g.). Although this is true for elemental carbon, it applies to organic carbon only in specific compounds (e.g., true carbohydrates) where it has an effective valence of zero. The oxidation of formic acid (CH_2O_2) and methane (CH_4) illustrates two divergent cases. One mole of each compound contains 1 gram atom (12 g.) of carbon; formic acid reacts with 1 gram atom (16 g.) of oxygen, and methane requires 4 gram atoms (64 g.) of oxygen. It is quite improbable that these researchers had samples with organic carbon existing at an exclusive, or even an average, valence state of zero (see the appendix). The point to consider is: while it is possible to interpret oxidation results in terms of carbon by assuming an average quantitative relation between C and O, it should not be assumed that a fundamental, invariable correlative exists between the quantity of oxygen required in a reaction and the amount of organic carbon present in a natural sample.

FACTORS AFFECTING THE EFFICIENCY OF DICHROMATE OXIDATION

Wet organic oxidation rates are finite; the rate and completeness of these reactions concern several factors whose effects are usually interdependent. It is necessary to study a procedure closely in order to adjust all possible conditions for maximum efficiency. The effects of these factors were either investigated by using the general procedure outlined previously or evaluated from literature reports. Since they do not operate independently, it was necessary to reinvestigate some factors in the light of later findings.

The oxidation potential: reagent concentration and temperature

A dichromate oxidation potential, in itself, does not express the rate of reaction; but for organic compounds in general, the higher the potential the more rapid will be the reaction. Reactant concentration and temperature are related in the simplified Nerst equation

$$E = E_o \frac{0.0001983\ T}{6} \log \frac{(H^+)^{14}\ (Cr_2O_7^{-2})}{(Cr^{+3})^2}$$

and can be considered together for convenience (see p. 6 for explanation of symbols).

Various reaction temperatures have been used in dichromate oxidations, the rate of reaction increasing with temperature. However, spontaneous decomposi-

tion of dichromate also increases with temperature, causing a definite loss in precision above about 150° C. Direct boiling methods require close adherence to a lower acid ratio (about 1:1) for this reason. Higher acid ratios may cause the mixture to explode if it is boiled directly. The temperature reached by a 2:1 mixture from heat of acid dilution (Allison, 1935; Walkley, 1935) is in the range of 115°-125° C., and is insufficient to oxidize all organic matter completely unless it is maintained by external heating. A boiling water bath continues the reaction with a negligible decomposition of dichromate.

Ames and Gaither (1914) are considered to be among the first investigators to get complete oxidation of soil organic matter with dichromate as a result of strong acid concentration (80-85 percent). Friedemann and Kendall (1929) studied the effect of water-acid relations on the oxidation of biological materials and clearly showed the superiority of reaction mixtures with low water contents. The situation has been reviewed more recently by Walkley (1947), who indicated a 2:1 volume ratio of concentrated H_2SO_4: aqueous dichromate. An optimal acid content is contingent, to a certain extent, on the procedure employed. Lower concentrations may achieve good results at high temperatures or with long reaction times. Higher acid concentrations precipitate dichromate (as chromic anhydride), nullifying the benefit of increased H-ion activity. The 2:1 acid:water ratio was adopted in this study. Precise measurement of acid is not necessary, but since H-ions are used in the reaction (also by the inorganic substances present) it is advisable to avoid falling below the specified amount of acid.

The effect of dichromate concentration on the oxidation potential is indicated in the Nernst equation (above). The initial concentration gives a specified potential that decreases as the reaction proceeds, according to the extent to which the dichromate becomes reduced. Consider first, the direct relation between the initial dichromate concentration and the effectiveness of oxidation as illustrated in figure 1. Three representative compounds were oxidized by the same procedure, except that the normality of the solution was varied. The concentration of reductant in each analysis was adjusted to react with 50 percent of the dichromate, assuming complete oxidation. Dichromate normality has been plotted logarithmically to stress the Nernst relation. It is evident that a practical lower limit of dichromate concentration must be selected in order to obtain reasonably good results. On the other hand, a maximum normality of 2.0 has been established in this procedure as the approximate solubility limit for dichromate in the reaction mixture. A dichromate normality of 0.05 has been chosen as the minimum for useful oxidation in the analyses described later.

Consider next the dichromate concentration at the end of the reaction. It has decreased in proportion to the amount of organic matter oxidized. Ideally, a constant dichromate concentration should be maintained throughout the reaction to expose all unoxidized organic matter to the same oxidation potential. On the other hand, the procedure must determine the change in dichromate concentration with reasonable accuracy and precision if the method is to be useful. Therefore, a sample of the proper size must be selected so that it reduces neither too much nor too little of the oxidant. These arbitrary limits are best decided by the particular objectives of the analysis. In general, one should strive for a reduction of about 50 percent of the dichromate. It may be permissible, in using 1.0 or 2.0 *N* dichromate, to reduce as much as 80 percent in a reaction. No more than 50

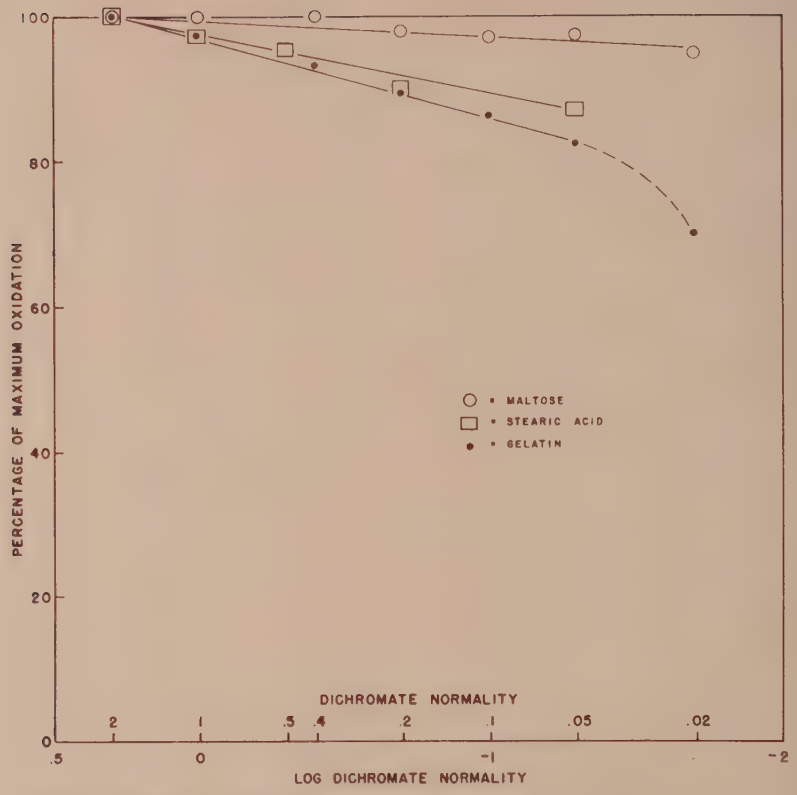

FIGURE 1.—Effect of different dichromate normalities, used in the same procedure, on the degree of oxidation of three organic substances.

percent of it should be reduced at low normalities, and acceptable results may be obtained, expecially when maximum possible oxidation is desired, with the reaction of only 5 percent of the dichromate present.

NATURE OF THE ORGANIC SUBSTRATE

Not all organic substances are oxidized with equal efficiency. Relative oxidation values for maltose, stearic acid, and gelatin, presented in figure 1, show that each of them reacts differently. This effect has been demonstrated for a wide variety of organic substrates, many of which are listed in table 2. These oxidations were accomplished over a period of many months by approximately the same procedure (the semi-micro method described in part IV). Some of the earlier analyses, especially of amino acids, were made before the procedure had been fully revised and would probably give slightly higher recoveries if reanalyzed. However, the purpose of this tabulation is to show only the general differences in oxidizability among the various organic compounds.

There appears to be a distinct relation among the oxidative susceptibility, the reduction state, and the general biochemical character of the organic substances. They have been arranged according to these similarities in table 2, and can be described oxidatively in terms of the common proximate groups.

Group 1. — The "carbohydrates": strongly hydrated or hydroxylated com-

TABLE 2.—*Comparison of theoretical and experimental Oxygen Equivalents of various organic substrates*

Substrate	Oxygen Equipment		Percent recovery
	Theoretical	Experimental [1]	
Group 1:			
Maltose	1.065	1.06	100
Starch	1.184	1.18	100
Cellulose	1.184	1.18	100
Oxalic acid	0.127	0.126	99
Sodium oxalate	0.119	0.119	100
Tartaric acid	0.533	0.531	100
Ammonium tartrate	0.434	0.434	100
Group 2:			
Glycerol	1.126	1.16	95
Stearic acid	2.925	2.85	98
Palmitic acid	2.870	[2] 2.63	92
Corn oil	[3] 2.85	2.77	97
Group 3:			
Uric acid	0.285	0.290	102
Cteatinine	0.677	0.628	93
Creatine	0.848	0.77	91
Uracil	0.713	0.717	101
Asparagine	0.727	0.615	90
Cystine	1.131	1.07	95
Glutamic acid	0.979	0.972	99
Glycine	0.639	0.55	86
Leucine	1.825	1.58	87
Methionine	1.501	1.03	69
Tryptophan	1.801	1.82	101
Tyrosine	1.681	1.66	99
Gluten	1.56 [3]	1.44	93
Zein	1.62 [3]	1.45	90
Gelatin	1.33 [3]	1.20	90

[1] All values are means of 3 or more analyses by the semimicro method with precisions within ±5 percent.
[2] Technical grade, O.E. probably low because of impurities.
[3] Calculated from molecular compositions given by Spector (1956).

pounds that have a characteristic O.E. of 1.2 or lower and are completely oxidized by dichromate.

Group 2.—The lipids: compounds essentially of carbon and hydrogen that have a characteristic O.E. above 2.0 and are almost completely oxidized (95-100 percent) by dichromate.

Group 3.—The crude proteins: various nitrogenous compounds that have an aggregate O.E. of about 1.5 in natural organic mixtures and are incompletely oxidized (90-95 percent) by dichromate.

Most other prominent compounds can be classified into this scheme, and a more elaborate treatment is given in the appendix.

The incomplete oxidation of most of these compounds can be considered a function of either their chemical resistance or their physical state. Since all of the substrates do react, these factors must influence the rate of reaction. In order to be oxidized efficiently, the substrate must be maintained in intimate contact with the oxidizing solution. A physical state which reduces this contact, such as that in particles or immiscible fluids, will therefore hinder the reaction. Proteinaceous particles and fatty materials are common examples. Microzonal reduced areas form at their interfaces, and they tend to float or adhere to the surface of the glass, where contact with dichromate is further reduced. These effects can be offset in part by agitating the reaction mixture, and it is advisable to do this with all samples as they are combined, and intermittently during the digestion period.

The resistance of protein compounds to oxidation is also partly chemical. Cellulose particles, for instance, react rapidly and completely; conversely, a gelatin suspension reacts very slowly. Since many of the amino acids also exhibit this resistance, it seems to be most characteristic of group 3. Much of the incomplete oxidation can be explained by the formation of acetic acid, which is very difficult to oxidize. The composition and configuration of many proteinaceous molecules is such that they tend to produce acetic acid in the process of decomposing. This seems especially true of alanine, and probably also of leucine and other resistant amino acids. The oxidation of such substances can be improved by using a catalyst as discussed in the following section.

REACTION TIME AND CATALYSIS

All the foregoing factors are manifest in the reaction rate between dichromate and organic matter. Their combined effects are demonstrated in figure 2 as time-oxidation curves for the three substances representative of the proximate-oxidation groups described earlier. Identical procedures were used in the analyses ("Semimicro method"), and the sample sizes were adjusted to re-

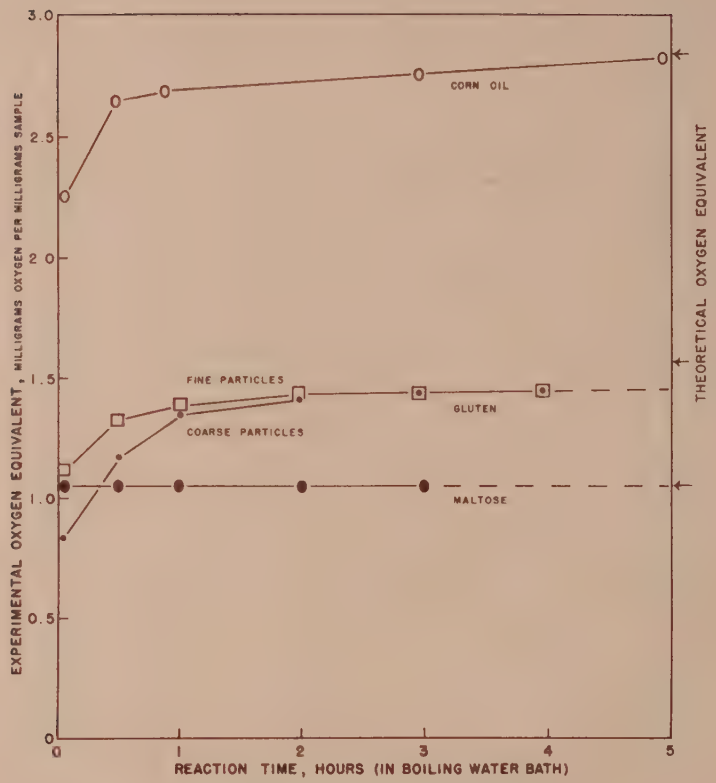

FIGURE 2.—Effect of reaction time on the completeness of oxidation of three organic substrates.

duce about 50 percent of the dichromate. Reaction time represents the number of hours each mixture was maintained in a boiling water bath. All of them were diluted and titrated at the end of 5 hours. Thus, at $t = 0$ the degree of oxidation is referrable to the reaction induced by heat of acid dilution, plus a minor amount of reaction which most probably occurred while the samples awaited analysis. In other words, if each sample had been diluted and titrated at the end of its specified reaction time, the curves would likely rise more sharply from a lower point on the ordinate.

Two curves are given (fig. 2) for gluten to show the influence of particle size on the rate of reaction. The sample material was roughly separated into two size groups by screening. "Fine gluten" was composed of particles about 0.2 mm. in diameter, and "coarse gluten" fragments ranged between 0.5 and 2.0 mm. The effect of surface area contact with the oxidizing solution is initially quite significant, but disappears at the end of about 2 hours' reaction time.

These curves can be considered characteristic of the oxidative response of the three proximate organic groups.

Group 1.—"Carbohydrates": The maltose curve is a straight line parallel to the X-axis at maximum theoretical O.E. Oxidation is complete with heat of dilution only and is not significantly affected by physical state because of strong oxidative susceptibility.

Group 2.—Lipids: The immiscibility of corn oil makes it difficult to oxidize. The reaction continues slowly with time and can be expected to reach completion eventually.

Group 3.—Crude proteins: Gluten is oxidized slowly and the curve levels off before maximum theoretical oxidation is attained. This effect is probably due to the loss of some unoxidized constituents.

From these data and other results not shown, an optimum reaction time of 3 hours in a boiling water bath has been selected. Although higher reaction temperatures can reduce this reaction time, the simplicity of a water bath and the reproducibility of results offset the temporal inconvenience. Incomplete oxidation, which is characteristic of certain compounds, is reproducible at any given dichromate concentration. It can be compensated for as an approximately constant error in any specified analysis.

A catalyst, silver sulfate, has been used in the preceding reactions, but its effect there has been slight. This is not true for all procedures. Lawton (1955) tested the efficacy of several metal ions as catalysts for dichromate oxidation using the Moore (reflux) technique. These findings indicated that silver was the most satisfactory catalyst, and was essential to the oxidation of acetic acid and its precursors. Many other researchers recommend its use. Christensen et al. (1937) and Van Slyke and Folch (1940), however, found catalysts ineffective in their oxidations. Friedemann and Kendall (1929) have pointed out the probable major cause of this discrepancy. They found that acetic acid was completely oxidized by a 1:1 ratio of acid:dichromate solution in the presence of Ag^+, but only 50 percent oxidized in its absence. Using the same procedure with 70-percent acid, they were able to oxidize acetic acid effectively without the catalyst. Thus, the catalytic effect of silver becomes less evident as the oxidizing conditions are made more rigorous. In the procedure employed here, silver sulfate increases the recovery of difficultly oxidizable substances by a few percent, at most. Considering that it produces no inimical effects in a strictly oxidative analysis, silver sulfate may well be included in the reaction mixture. It is conveniently added as a solute in the acid used.

Interferences

A number of other factors which operate to increase or decrease the observed Oxygen Equivalent from its true value are classified together here as interferences. They may be severe in special instances, but usually can be avoided or compensated in the final analysis.

Oxidizable inorganic substances can give erroneously high results if not properly regarded. A sample taken from an aerobic situation is not likely to contain dichromate-oxidizable inorganic compounds with the exception of halides, chlorides in particular. Chloride is oxidized to free chlorine, and the presence of even minute amounts in a reacting mixture is detectable from the odor of Cl_2 at the mouth of the flask. This reaction is not rapid, and is complicated by the formation of chromium oxychloride (CrO_2Cl_2), which is volatile and may be observed as a dark-red liquid adhering to the upper, inner surfaces of the reaction flask. Soluble chlorides are precipitated as AgCl if silver sulfate is included in the reagents. Some reports imply that chloride is not oxidized from the silver precipitate (Kay, 1954; Nikolaeva, 1953; Peech et al., 1947), but others indicate that it is at least partly oxidized (A.P.H.A., 1956; Buydens and Ledent, 1957). The opposing views apparently result from different procedural effects.

The oxidizability of chloride as Cl^- and AgCl was tested by the procedure employed in this study. After 1 hour's reaction time, 73 percent of the dissolved chloride and 68 percent of the precipitate had been oxidized. At the end of 3 hours, nearly all of the AgCl was oxi-

dized, but the Cl^- recovery increased only slightly (perhaps owing to retention of undecomposed chromium oxychloride). A further observation was that Ag_2SO_4 seemed to preclude the formation of CrO_2Cl_2, which would be a benefit additional to its catalytic function.

The presence of small amounts of chloride in a sample (about one-tenth the weight of organic matter) usually can be disregarded because the Oxygen Equivalent of chloride (0.226) is less than one-sixth that of "average" organic matter. A sample Oxygen Consumed value can be corrected for moderate amounts of chloride by quantitatively analyzing for Cl^- in a duplicate sample aliquot and subtracting the calculated chloride O.C. (chloride O.E. $\times$ mg. present) from the total O.C. If the chloride O.C. exceeds the organic O.C. in a sample, other methods of adjustment will be necessary in order to obtain a reasonably accurate quantitative organic oxidation.

A sample from an anaerobic milieu can be expected to contain other oxidizable inorganic elements, especially ferrous iron and reduced sulfur. These may be serious interferences, for instance, in samples of lake muds. Reduced inorganic elements become oxidized in contact with atmospheric oxygen. This might occur sufficiently during sample treatment (drying, grinding, storage) to remove the interference. Possibly, the sample could be preoxidized with a mild reagent to convert the reduced inorganic matter before quantitatively oxidizing it with dichromate. A confounding factor is the presence of unstable organic compounds which partly oxidize in contact with the atmosphere. The quantitative oxidation of samples from highly reduced environments appears to be feasible, but the correct interpretation of such results requires considerations beyond the present treatment.

The most common analytical interference in a quantitative oxidation is the presence of contaminants. The general precautions of microanalysis are applicable here, especially in regard to reagent purity and cleanliness of glassware. Reaction flasks should be cleaned with chromic acid and kept covered to avoid direct contact with the atmosphere. For critical studies, special redistilled water (Medalia, 1951) and recrystallized dichromate (Kolthoff and Sandell, 1952) are advised. The reagent blank compensates for some, but not all, contamination that may occur.

The loss of unoxidized substances and the spontaneous decomposition of dichromate are two interferences that have been mentioned previously. A reflux condenser can eliminate the loss of most volatile matter, but it also complicates the procedure. The use of a suitable reaction flask minimizes such losses; Kjehldahl flasks are excellent in this respect, but cannot be used with all types of samples. Regardless of the kind of flasks used, they should be immersed in the water bath only at the base, so that the upper portions remain cool and permit condensation to occur. Spontaneous decomposition of dichromate cannot be measured independently from reduction by contaminants in the reagents but appears to be negligible in this procedure. The total loss of dichromate in the absence of an intentional reductant increases logarithmically with reaction time, as evidenced by reagent blank titers. The observed magnitude of this loss in a reaction period of 3 hours is usually less than 2 percent with 1-2 *N* dichromate and less than 5 percent with 0.05 *N* dichromate. If losses much higher than these are encountered, the analyses should be viewed with suspicion.

Part IV. METHODS OF DICHROMATE OXIDATIVE ANALYSIS

The diverse nature of limnological samples and their wide range of organic concentrations each impose special methodological considerations. First, the general procedure requires a sample of a certain physical state, and the validity of analysis requires that the organic matter in the sample be as little changed as possible from its original state. The treatment given to a sample before analysis, therefore, is no less important than the analysis itself. Second, the general procedure was selected partly because of its usefulness over a broad analytical range. To accomplish this effectively for a variety of samples, a few minor procedural adjustments are necessary. Two specific methods of oxidation are described below which apply to the most common sample types: particulate matter that is available in weighable amounts, and small quantities of dissolved substances that are meted in terms of water volumes. Analytical adjustments necessary for the extension of these methods to samples of other types and sizes are suggested.

PREANALYTICAL SAMPLE TREATMENT

The technique of oxidative analysis actually begins with sampling, but a discourse on this subject is beyond the present objectives. One word of caution is offered: the results obtained by oxidative analysis will be no better than the care and consideration given to the taking of representative samples. The following discussion, then, refers to samples which have already been secured. It is convenient, although somewhat factitious, to consider the initial form of a sample as either a weighable particulate mass or material dispersed in a large quantity of water. The former refers to such as filter or centrifuge residues, sediments, periphyton, benthic fauna, and even macrophyte or vertebrate tissues; the latter concerns only water samples, with or without dispersed particulate matter.

Solid materials usually are analyzed on a weight basis, but sometimes a volume (e.g., plankton), surface area (e.g., periphyton) or numerical (e.g., individual invertebrates) basis is preferable, especially if the sample is analyzed fresh. Samples intended for later analysis are conveniently preserved by drying, which is best done at low temperatures in a vacuum. Lyophilization probably is a better method. Dried samples frequently require grinding in a mortar to reduce them to fine particles; this process also increases homogeneity. A dried, ground sample should be stored in a closed vial.

An Oxygen Consumed value can be determined for any sample by quantitative oxidation, as previously discussed, but an Oxygen Equivalent can be estimated analytically only if the organic weight of a sample is known with reasonable accuracy. Obtaining the O.E. of a weighable sample is an important consideration, for it permits a better characterization of the organic matter present. Natural samples contain mineral matter and water in addition to organic compounds, and the proportionate weight of these inorganic constituents can be determined for many samples by careful drying and ignition. Organic matter is notoriously hygroscopic; analysis on an air-dry basis alone is not always satisfactory, even for comparative oxidations. Birge and Juday (1922) described the difficulty of removing residual moisture from plankton material and found that dried samples stored in bottles for prolonged periods lost up to 7 percent of their weight upon redrying. Similar experiences were encountered in this study (1 to 3 percent moisture gain in vacuum-dried samples

during storage for several months), and it was considered necessary to redry all stored samples at the time of analysis. Probably it is impossible to remove all traces of water from organic matter; the moisture content is merely reduced to an insignificant level. Determination of the mineral content of moisture-free material by ashing is subject to error (see p. 878 in Hutchinson, 1957) and can be used in the approximation of true organic content only when the proportion of mineral in the sample is relatively low. The ash content of samples reported here was measured by igniting the weighed dry samples at about 600° C. for a few hours, rehydrating the cooled residues with a few drops of distilled water, redrying at 100°-105° C., and weighing them again after they had cooled in a desiccator.

The treatment of water samples presents a different sort of problem. Efficient oxidation depends on a low water content in the reacting mixture. Except in unusual cases of high organic content, water samples must be concentrated for analysis. Ordinarily, between sampling and concentration there is a significant time lapse during which oxidative decomposition might occur. Oligotrophic lake waters, for instance, may contain more than twice the amount of free oxygen needed to oxidize all of the organic matter they contain. Skopintsev (1949b) has observed that the organic content of untreated river water decreases logarithmically during storage at 16°-20° C. The initially rapid rate of loss, attributed to labile components, became slower and nearly constant after 40 to 50 days. Not all such oxidative decomposition is microbial. Krogh and Lange (1932) found that dark-stored lake water, apparently free of microorganisms, consumed a few milligrams of oxygen per liter during the course of a month. Autolytic transformations in bacteria-free algal sediments were studied by Aleyev (1934), who demonstrated that lysis proceeds slowly but the causative enzymes retain their activity for a long time. Ultraviolet radiation also promotes the reaction between dissolved oxygen and organic matter (Drachev and Mityagina, 1957).

Organic matter from waters normally saturated with oxygen may be more stable than that indicated above. In this study, there was no detectable change in the organic content of oligotrophic lake water stored dark, sealed, and refrigerated for periods up to a few weeks. Preventive treatment may be necessary for labile samples or for prolonged storage. Frozen storage or removal of free oxygen (as by flushing with N_2 or CO_2) might prove satisfactory. Acid fixation in the field, as is frequently used in marine research (Duursma, 1960; Kay, 1957), is another possible method. Organic fixatives, such as toluene or chloroform, should not be used since they are never removed completely from the sample, even when it is evaporated to dryness. The oxidative decomposition of organic matter in water requires further study; the safest practice to follow in the quantitative oxidation of water samples is to analyze them as soon as possible after they have been taken (see Strickland and Parsons, 1960, p. 113).

A water sample is most frequently concentrated for analysis by evaporation in an oven at 100°-105° C. (e.g., Krogh and Keys, 1934). Nikolaeva (1953), however, selected 60°-70° C. as optimum for evaporation and also neutralized the water with sodium carbonate to prevent the loss of volatile acids. In the present investigation, 50–250 ml. aliquots of water were evaporated from widemouth Erlenmeyer flasks, which also served as oxidation vessels. The effect of evaporation temperature on Oxygen Consumed values of oligotrophic lake water was tested. Triplicate portions of two samples, one filtered (milli-

pore HA) and one unfiltered, were evaporated to dryness in a thermostatically controlled electric oven at various temperatures. The data are shown in figure 3. Temperature of oven operation ranged within ±3 degrees of the selected value. The difference in relative displacement of the two curves indicates the O.C. of seston contained in the unfiltered sample. The shapes of the curves suggest a difference in response to drying temperature. Unfiltered water residues consumed most oxygen after drying at about 90° C., but the residues of filtered water dried at 78° C. showed the greatest response to oxidation. These findings indicate an optimum drying temperature range for this water of 80°-90° C. The effect of drying temperature on maximum organic retention is not known for other waters but is expected to vary.

Concentration of water samples by drying at elevated temperatures is obviously unsatisfactory for critical studies because of the uncertain losses and probable chemical changes of the organic matter. Drying at intermediate temperatures requires a long time, during which enzymatic oxidations evidently occur. Freeze-drying probably is most efficacious but requires special apparatus and is difficult to apply to large volumes of water. Freeze-concentration may prove to be a very useful technique for studies concerning dissolved and dispersed organic matter.[1]

[1] Simple, slow freezing is effective in concentrating the solutes from water. An 8-liter sample of filtered pond water was slowly reduced to 1 liter of liquid in a deep-freeze unit. The separated phases gave the following evaporation (T.D.S.) data: Liquid, 542 mg./L.; ice, 6 mg./L. See also pages 98–99 in Ellis et al. (1948), page 170 in Welch (1952), and Shapiro (1961).

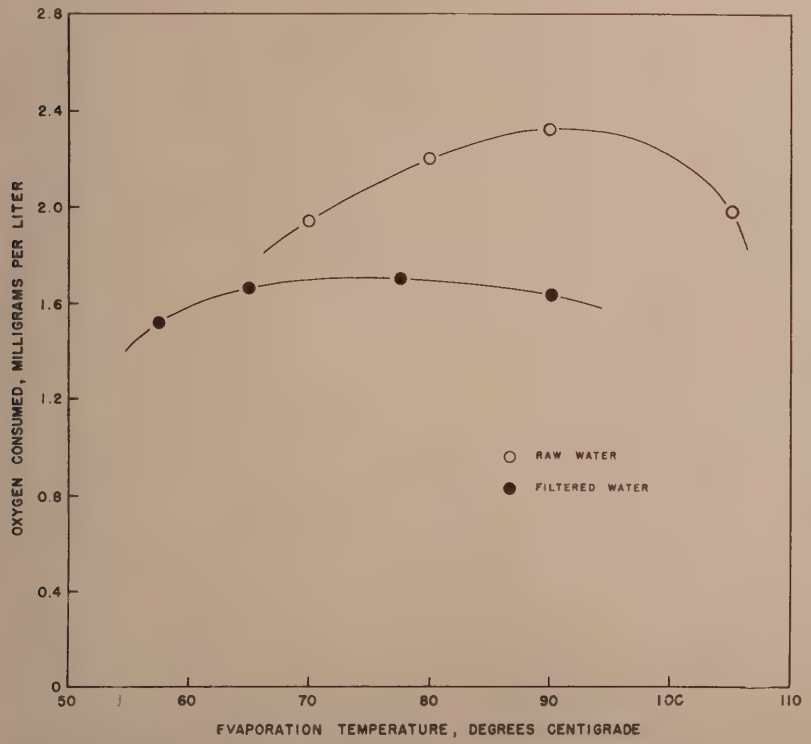

FIGURE 3.—Effect of evaporation temperature on the oxygen consumed by raw and filtered lake-water samples.

QUANTITATIVE OXIDATIONS

Semimicro method

A technique was needed at first to investigate the oxidizability of purified organic compounds, and later to characterize the particulate matter (e.g., plankton) from the aquatic environment. Its principal requirement was that it be applicable to the smallest samples accurately weighable on a standard analytical balance. The following categorical procedure concerns a dry sample requiring about 40 mg. of oxygen for complete oxidation (which represents about 25–30 mg. dry, ash-free biological material). However, the method is broadly applicable to samples that are dry, moist, or in solution, and which may consume from about 2 to 100 mg. of oxygen (about 2–70 mg. natural organic matter). The extended range of analysis is accomplished by minor alterations of reagent volume or concentration which are discussed subsequently.

1. Reagents:

a. Standard potassium dichromate, 2 *N*: Dry 100 g. reagent grade $K_2Cr_2O_7$ crystals at 150° C. for 2 to 3 hours, cool in a desiccator, weigh out and dissolve 98.07 g. in distilled water, and make up to 1 liter.

b. Ferrous sulfate, approximately 1 *N*: Dissolve about 280 g. $FeSO_4 \cdot 7H_2O$ in distilled water, add 20 ml. concentrated H_2SO_4 and make up to 1 liter. It is advisable to filter this solution and store it in a stoppered brown-glass bottle. Standardize, when used, against the dichromate solution. One-half normal ferrous ammonium sulfate can be employed alternatively, but higher normalities may result in a supersaturated solution from which the salt precipitates during storage.

c. Sulfuric acid, concentrated (96 percent or more).

d. Phosphoric acid (ortho-85 percent).

e. Silver sulfate: Dissolve 12 g. Ag_2SO_4 in each liter of H_2SO_4.

f. Barium diphenylamine sulfonate (indicator): Prepare a 0.3-percent solution by dissolving 0.15 g. in 50 ml. warm distilled water.

2. Glassware:

a. Reaction flasks: 30 ml. Kjehldahl or round-bottom boiling flasks of a similar size.

b. Titration flasks: 250 ml. Erlenmeyer.

c. Volumetric ware: one transfer pipette, 5 ml.; one burette, 10 ml. (semimicro), calibrated at least to 0.05 ml.; two graduated cylinders, 10 ml. and 200 ml.

3. Procedure:

It is expeditious to oxidize a series of samples or sample replicates simultaneously. At least one reagent blank should accompany each series through the entire operation (steps b through g). The following procedure applies to any member of the series.

a. Weigh out a sample which will require 20–50 mg. oxygen (e.g., about 30 mg. dry algal material) and place it in the bottom of a reaction flask. This may be done conveniently with a long-shanked weighing scoop (see Van Slyke and Folch, 1940) or by weighing the sample into a small, clean glass cup which can be admitted and left in the reaction flask.

b. Pipette 5 ml. standard dichromate into the flask, allowing the solution to run down the inner surfaces of the neck as the flask is rotated. Swirl gently to disperse the sample in the dichromate.

c. Measure about 11 ml. concentrated sulfuric acid into a graduated cylinder (1 ml. excess is allowed here for the acid which adheres to the sides of the cylinder). Add rapidly to the reaction flask while swirling it gently. The re-

action progresses immediately upon acidification, and swirling should be continued for about 30 seconds.

d. Stopper the flask lightly with a glass or polyethylene plug and immerse the base in a boiling water bath so that its neck is cooled by ambient air. Note well: This mixture should not be boiled directly nor exposed to a higher temperature for prolonged periods.

e. Maintain the flask in the bath for 3 hours, swirling it at about half-hour intervals. There should be no organic particles observable in the flask after 2 hours. Before the final swirling, hold the flask at a steep angle and rotate it to rinse the lower part of the neck with the oxidizing mixture. Remove the flask from the bath after 3 hours and cool slightly.

f. Transfer the contents to a titration flask, rinsing it out with several successive portions of distilled water. Dilute the mixture to about 150 ml.

g. Cool the diluted solution to room temperature, add 5 to 10 ml. phosphoric acid and 2 or 3 drops of indicator. Titrate with the ferrous solution. The endpoint is very sharp and reversible, changing from a red or reddish blue to bright green. The reagent blank will require about 10 ml. 1 *N* ferrous solution and the samples proportionately less, according to organic content.

h. Since the ferrous solution requires standardization, it is conveniently done in duplicate at the time samples are titrated. Pipette two 5-ml. aliquots of 2 *N* dichromate into clean titration flasks, add about 150 ml. distilled water and 5 ml. sulfuric acid to each, mix, cool, add 10 ml. phosphoric acid and 2 drops indicator. Titrate each as described above.

4. Calculations:

a. Standardization of reductant:

$$\text{Normality } Fe^{+2} = \frac{\text{ml. dichromate} \times \text{normality dichromate}}{\text{ml. } Fe^{+2} \text{ solution}}$$

b. Oxygen Consumed: O.C. = (reagent blank titer − sample titer) × *N* Fe^{+2} × 8, with titer volumes in milliliters. This equation gives the milligrams of oxygen the sample required; divide by the sample weight (mg.) to express O.C. as a weight ratio.

c. Oxygen Equivalent: If the sample is purely organic, the O.C. (as weight ratio) represents the experimental O.E.; if it is partly organic, an experimental O.E. is calculated on a moisture- and ash-free basis. Exper. O.E. =

$$\frac{100 \times \text{O.C. (as wt. ratio)}}{100 - (\% \text{ moisture} + \% \text{ ash})}.$$

5. Example:

Samples of three plankton organisms were oxidized by the semimicro method, and this data is presented in table 3. The two genera of phytoplankton and one of zooplankton were obtained in a relatively pure state from Crowley Lake, Calif., by screen filtrations of localized concentrations. The analysis was done in replicate in two series. The only departure from the outlined procedure was the use of 1 *N* dichromate (Fe^{+2} normality and weights analyzed were proportionately lower). The precision of the replicates, expressed as both coefficient of variation and percentage average deviation, is generally within ±1 percent. The higher coefficient of variation for *Aphanizomenon* is probably caused by heterogeneous sample material, since it was not ground as fine as the other two substrates. The interpretation of these oxidation values will be discussed later.

6. Comments:

To extend this method over a wider range of sample sizes without significantly changing the effectiveness of oxidation requires only slight modification. Samples needing up to 100 mg. oxygen (or containing up to 80 mg. organic matter) can be analyzed by doubling the reagent volumes and by using 100-

TABLE 3.—*Oxidation analyses of plankton by the semimicro method*

Series	Sample		Ferrous solution, milliliters [1]		Oxygen Consumed, milligrams	
	Plankton organism	Air-dry weight (mg.)	Titer	Sample equivalent	Per sample	Per milligram of sample
A – 1	Reagent blank	0	9.76			
2	do	0	9.76			
3	*Aphanizomenon*	16.0	4.86	4.90	19.90	1.244
4	do	21.1	3.41	6.35	25.79	1.222
5	do	18.9	4.05	5.71	23.19	1.227
6	*Daphnia*	13.5	6.26	3.50	14.21	1.052
7	do	21.6	4.27	5.49	22.30	1.032
8	*Gloeotrichia*	17.3	4.60	5.16	20.96	1.212
B – 1	Reagent blank	0	9.81			
2	do	0	9.81			
3	*Daphnia*	18.4	5.15	4.66	18.89	1.027
4	do	18.1	5.22	4.59	18.61	1.028
5	*Aphanizomenon*	22.6	3.14	6.67	27.04	1.196
6	do	18.9	4.12	5.69	23.07	1.221
7	*Gloeotrichia*	19.3	4.04	5.77	23.39	1.212
8	do	20.1	3.81	6.00	24.32	1.210

Analytical summary:		*Aphanizomenon*	*Daphnia*	*Gloeotrichia*
Oxygen Consumed:				
Mean value		1.22	1.04	1.21
Average deviation	percent	±0.9	±0.8	±0.1
Coefficient of variation	percent	±1.4	±1.0	±0.1
Inorganic content:				
Ash	percent	4.7	19.4	4.0
Moisture	percent	4.2	6.3	5.5
Oxygen Equivalent		1.41	1.46	1.41

[1] Normality = 0.507.

ml. Kjehldahl flasks. Dilution for titration must be increased correspondingly. The analytical range can be lowered similarly by decreasing reagent volumes and flask sizes, or by using 1 *N* dichromate (as in the above example) which is minimum for good efficiency and precision. A sample containing about 2 mg. organic matter can be analyzed satisfactorily using 1 ml. of 1 *N* dichromate and 2 ml. acid in a test tube.

This method is not restricted to dry samples. Moist or aqueous material of suitable organic content can be analyzed if attention is given to the minimum dichromate normality and proper acid: water ratio (2:1). To oxidize an aqueous sample, dichromate can be used in a dry state (e.g., pipette the required amount of standard dichromate into an empty reaction flask and evaporate to dryness before adding the sample solution). The precision shown in the preceding example is about average for natural samples. Pure organic compounds can be oxidized by this method to within ±0.5 percent of the mean value, if the procedure is followed with care (cf., exper. O.E.'s. in table 5). The reagent blank titer should be within 3 percent of the standardization titer.

Micro method

The following method was designed to analyze samples of natural waters, especially those which are low in organic content. It is actually only a variation of the preceding method, differing in strength of reagents and in sample manipulation. The procedure outlined is optimal for samples requiring slightly less than 1 mg. oxygen (or, samples containing from 0.07 to 0.7 mg. organic matter). However, it can be used on natural waters throughout their range of organic concentration without procedural alteration because the sample aliquot is easily adjusted to optimum reaction level.

1. Reagents:

a. Standard potassium dichromate, 0.05 *N*: Prepare by diluting 2 *N* dichromate, or dissolve 2.452 g. dry, reagent grade $K_2Cr_2O_7$ in distilled water and make up to 1 liter.

b. Ferrous sulfate, approximately 0.03 *N*: Either dilute the 1 *N* solution and acidify to 2 percent H_2SO_4 by volume, or dissolve 8.4 g. $FeSO_4 \cdot 7H_2O$ in distilled water, add 20 ml. concentrated H_2SO_4, cool, and dilute to 1 liter.

c. Other reagents as in the semimicro method: Concentrated sulfuric acid with silver sulfate, concentrated phosphoric acid, barium diphenylamine sulfonate indicator.

2. Glassware:

a. Combined evaporation-reaction-titration vessels: 250 ml. widemouth Erlenmeyer flasks with glass or polyethylene covers.

b. Volumetric ware: All items listed in the semimicro method, plus two transfer pipettes, 50 and 100 ml.

3. Procedure:

It is advantageous to treat a series of four or more samples concurrently. The following operations describe a single member of the series. At least one reagent blank, for which step a is omitted, must be included with the series.

a. Pipette an exact volume of sample water, which will require from 0.1 to 1 mg. oxygen, into an evaporation flask. Normally, this will be between 50 and 100 ml., and is conveniently considered in 50-ml. steps. If the approximate oxygen requirement of the water is unknown, use a 50-ml. aliquot in a pilot analysis and adjust to an optimum level, if necessary, in subsequent analyses. Evaporate the sample to dryness at no more than 100° C. (see page 19), taking precautions against contamination from the atmosphere.

b. Proceed exactly as in steps b through e, g, and h of the semimicro method, except use the reagents specified here. The reacted mixture is not transferred to a titration flask. Instead of semimicro step f, remove flasks from the bath and dilute contents in situ with 100 ml. distilled water.

4. Calculations:

a. Standardization of ferrous solution: Use formula in semimicro method.

b. Oxygen Consumed: Mg. O.C. is calculated as in semimicro method. Since the samples here are analyzed on a volume basis, the expression of O.C. as a ratio is calculated on a liter basis. O.C.

$$\text{in mg/L.} = \frac{\text{O.C. in mg.} \times 1000}{\text{ml. sample used}}.$$

5. Example:

Table 4 contains the results of analyses of 1 filtered and 2 unfiltered oligotrophic lake water samples, 4 determinations (of 200 ml. aliquots) having been made on each. Raw mean water and Filtered mean water are from the same original sample. The difference in their O.C. values is the O.C. of the seston. In general, the precision shown is about ±4 percent as the average deviation, and about ±5.5 percent as the coefficient of variation.

6. Comments:

Using a pure, easily oxidized compound such as sugar for a substrate, a precision of better than ±2 percent is attainable. Lower precision for raw water is a common phenomenon apparently due to chance occurrence of from none to a few macrozooplankters in any given aliquot. One aliquot without a *Daphnia,* for instance, may yield an O.C. of 0.5 mg/L. A second aliquot, identical to the first except for the presence of two 2-mm. *Daphnia* (with eggs, dry weight = 0.12 mg.; Richman, 1958), would have an O.C. of 0.62 mg/L. (based on data of table 3), or differ by more than 20 percent. Several replicate analyses may be needed to reach a valid mean O.C. in such cases. For this method, reagent blank titers should be within 5 percent of standardization titers. If they differ more, the procedure should be examined for possible sources of contamination.

TABLE 4.—*Quantitative oxidation of oligotrophic lake water*[1] *by the Micro Method*

Aliquot	Oxygen Consumed, milligrams					
	Raw mean water [2]		Filtered mean water [2]		Raw mean-depth water	
	Per aliquot	Per liter	Per aliquot	Per liter	Per aliquot	Per liter
No. 1	0.566	2.83	0.500	2.50	0.614	3.07
No. 2	0.644	3.22	0.560	2.80	0.524	2.62
No. 3	0.610	3.05	0.550	2.75	0.568	2.84
No. 4	0.600	3.00	0.538	2.69	0.578	2.89
Mean Oxygen Consumed		3.03		2.69		2.86
Average deviation		±3.7%		±3.4%		±4.4%
Coefficient of variation		±5.4%		±4.9%		±6.5%

[1] Convict Lake, Calif., 9 July 1959. Mean depth 52 feet. All analyses were made by use of 200-milliliter aliquots.
[2] A mixture of equal water volumes taken from 5 strata selected to represent the lake average.

It is difficult to increase the sensitivity of this particular method for two reasons: First, lowering the dichromate normality is not advised because of reduced oxidative efficiency (see part III); second, the volume of the reaction mixture needed to cover the inner surfaces of the flask adequately is about 12 ml. (4 ml. Cr^{+6}, 8 ml. acid), below which recoveries become too devious. However, a simple micro method, patterned after the suggestion on page 22 but using 0.05 *N* dichromate, can be devised for special samples (particles or solutions up to 2 ml. in volume). A single *Daphnia*, as described above, can be quantitatively oxidized by 1 ml. of 0.05 *N* dichromate and 2 ml. acid in a small reaction tube. The micro method can accommodate larger amounts of organic matter than is stated, by employing more concentrated solutions of dichromate. If water contains more than 40 mg. organic matter per liter, a convenient approach would be to evaporate the dichromate in a reaction flask (see page 22), add enough sample water to reconstitute a 0.05 *N* solution, and acidify at the 2:1 ratio.

Discussion

The methods presented here are proposed not as limnological standards but rather as introductory approaches to a broader investigation of natural organic matter. Their function in limnological research will become more apparent in the following treatment. The immediate concern is the improvement in the quantitative oxidation technique, especially in regard to time and completeness of oxidation. Moreover, the basic procedure may not fit the needs of other particular problems. To this end, some final methodological explanations may be of assistance.

1. Reagents and titration:

Dichromate solutions are extremely stable until reacted and require only normal care in handling. Ferrous solutions are rather unstable under some conditions. They are acidified (about 2 percent acid by volume) to increase stability and storage life. Actinic and temperature factors also affect stability but if ferrous solutions are stored in stoppered, brown-glass bottles in a cool place, their titer loss after a couple of months amounts to less than 10 percent. When used daily in the same manner, the normality of ferrous solutions was observed to decrease at a constant rate (about 0.5 percent per day). This suggests that daily standardization may not be necessary in an uninterrupted series of analyses.

Titration indicators are organic compounds and are susceptible to decomposition by strong acid and dichromate concentrations, and even weaker oxidiz-

ing solutions at high temperatures. Thus, it is necessary to dilute and cool the reaction mixture. Dilution also serves to reduce the color intensity of dichromate-chromic ions, giving the requisite transparency for titration. Phosphoric acid is added to complex ferric ions formed during the titration, thus lowering the ferrous-ferric system potential to give a better endpoint with the diphenylamine sulfonate indicator. The color change at the endpoint is complex because the color observed at any point is the result of both indicator and chromium compounds. The exact shade varies with dilution and relative concentrations of the color-producing ions. Chromium ions change from orange to bright green through continuous transitional intermediates. Superimposed is the indicator color, a reddish violet in the oxidized state and colorless when reduced. The endpoint change is always sudden and sharp, even in dilute solutions as a few trial titrations will demonstrate. Back titration is possible but not necessary after a little experience is gained. If the endpoint is consistently overshot, the analyst is advised to reserve a few milliliters of the untitrated mixture in a clean container, rinse this into the titration flask when the initial endpoint is exceeded, and proceed cautiously to the final endpoint.

There are other satisfactory indicators for this titration which are discussed in analytical texts (e.g., Kolthoff and Sandell, 1952). The excess dichromate also can be determined colorimetrically by measuring the absorbance of the reaction mixture at 645 millimicrons and comparing it to a standard absorption curve (for details, see Graham, 1948).

2. Glassware and other equipment:

The glassware listed above is considered most suitable for these methods, but other items may be substituted or added to the list. The only essential piece of volumetric ware for austere circumstances is the burette. It may be used first to deliver the necessary volume of dichromate, rinsed, and then employed in the titration. Test tubes or small beakers, calibrated with the burette, can be used to measure out the acids.

Accessions which can expedite a large number of concurrent analyses are a reservoir burette and a magnetic stirrer. The latter is especially functional since adept endpoint detection depends to a large extent on how well the solution being titrated is kept mixed.

Inner surfaces of the pipette or burette used to dispense the dichromate solution become "dry" after a short time and require cleaning. This can be done by flushing the item with dilute hydroxide, followed by a rinse with distilled water.

3. Analytical precision and accuracy:

Both methods are capable of higher precision than shown in the examples, by using pure compounds as reductants and by taking special care in the preparation and measurement of reagents. It seems pointless to demonstrate this, however, since these methods are intended for the analyses of natural samples whose organic heterogeneities usually impose separate limits on the precision attainable. Samples of filtered water frequently replicate with high precision, but this is not always true. The drying process apparently exerts a major influence on reproducibility. Samples used in examples above were selected as representative of the limnological material to which these methods are applicable, and the stated precisions (semimicro method $= \pm 1$ percent; micro method $= \pm 5$ percent) are considered to be of a corresponding representative order of magnitude.

The accuracy of these methods on natural substrates is difficult to assess. It

was pointed out in part III that some compounds are completely oxidized, while others give various incomplete recoveries (see table 2 and figs. 1 and 2). For any specific compound, the proportion unoxidized can be considered an almost constant error and therefore can be adjusted. Since there are a great many organic compounds in a natural sample, certain assumptions are necessary in adjusting its observed oxygen requirement to a "true" value. This has been done through the medium of proximate groups, which are discussed in the appendix. It is presently assumed that the oxidation of natural organic matter is 95 percent complete by the semimicro method, and 85 percent complete by the micro method. In using either method, directly or with modification, it is advisable to check the accuracy occasionally by means of "standard reductants". The absolute accuracy is best measured by using primary standard grade sodium oxalate. A convenient relative standard having oxidative stability is gelatin, which can be weighed in a dried form or pipetted from a "solution" of known concentration.

The presence of inorganic chloride in a sample will also affect the accuracy, and corrections must be made in the final results. It has already been suggested that, at interfering levels, the chloride content be determined and its Oxygen Consumed value (0.226 $\times$ wt. Cl^-) subtracted from the sample O.C.

4. Modifications and other oxidation procedures:

Several adjustments have been mentioned which extend the two methods to a variety of sample sizes and types. If the characteristics of the analyses described here are to apply to a modified procedure, three considerations are of particular importance:

a. An acid:water ratio of not less 2:1 by volume must be employed.

b. The stronger the dichromate concentration (up to about 2 *N*), the more effective will be the oxidation. Dichromate solutions less than 0.05 *N* should not be used.

c. The degree to which any sample is oxidized depends on the time-temperature relation of the reaction.

There are other dichromate oxidation procedures which may be useful. Those employing higher reaction temperatures can give a more rapid analysis. One of the simplest is Tiurin's (1931), which utilizes a lower acid:water ratio (about 1:1) in a 5-minute, direct-boiling reaction. A rapid procedure of this type may be desired in certain instances, but many of the characteristics that have been described here will not apply.

DETERMINATION OF ORGANIC ELEMENTS

Oxidative decomposition of organic compounds by acid dichromate in the methods just described differs only in minor respects from the digestions or combustions employed in common ultimate analyses. The end products are the same; Kjehldahl digestion producing NH_3 from organic nitrogen, and dry combustion achieving the transformation of organic carbon to CO_2. These inorganic forms of the two most important organic elements lend themselves to a variety of existing quantitative analyses. The problem posed was to find simple, direct techniques which could be incorporated into the dichromate oxidation scheme. Separation of CO_2 and NH_3 from the reaction mixture by diffusion and estimation by titration, as described below, appear to be the most suitable ones. These direct analyses can be complemented with a mathematical determination of hydrogen and oxygen in order to characterize all of the major elements in organic matter. The necessary calculations will be considered in part V.

Carbon analysis

Carbon dioxide is freely evolved from the acid oxidation mixture as the reaction progresses. There are many published methods treating the absorption and measurement of CO_2, and one of the simplest is that described by Baker et al. (1954). In essence, it is a nonquantitative wet oxidation in which a sealable jar serves as a combined combustion-diffusion vessel. Evolved CO_2 diffuses into an internally contained hydroxide solution and is measured after the reaction subsides by double titration with HCl. This procedure has been modified by Katz et al. (1954) for greater precision, and by Chen and Lauer (1957) for use with water-soluble materials. The following is adapted, in part, from the above papers. Since this determination becomes part of the quantitative oxidation procedure, its analytical range is limited by the size of the sample which can be used in a given oxidation. The oxidation procedure of the semimicro method is employed, so that the corresponding range of carbon detection is 5 to 20 milligrams. Microdetermination of carbon is possible by an appropriate adjustment of the absorbent concentration.

1. Apparatus and reagents:

All reagents and glassware listed for the semimicro method are required with the exception of the oxidation flasks. Below are the additional items needed for carbon absorption and titration.

a. Reaction-diffusion vessels: Erlenmeyer flasks, 125 to 250 ml., having single-hole rubber stoppers fitted with short lengths of rubber tubing and clamps. Alternatively, 250 ml. vacuum flasks with solid stoppers and clamped outlets may be used. Coat all stoppers very lightly with silicone grease.

b. Sample holders: 5-ml. beakers or similar flat-bottom glass cups which can be inserted into the reaction vessels.

c. CO_2 absorption tubes: Shortened test tubes or long vials, about 20 mm. in diameter, which fit up to (but not into) the neck of the reaction vessel when inserted.

d. Titration flasks: 125-ml. Erlenmeyer flasks with stoppers.

e. CO_2-free sodium hydroxide, approximately 2 *N*: prepare as needed from saturated stock (about 55 g. NaOH/100 ml. distilled water) by diluting about 16 ml. clear supernatant to 100 ml. with CO_2-free water.

g. Barium chloride, approximately 2 *N*: Dissolve 25 g. $BaCl_2 \cdot 2H_2O$ in 100 ml. CO_2-free water and store in a stoppered flask. If a precipitate forms, use only the clear supernatant solution.

h. Standard hydrochloric acid, 0.2 *N*: Dilute 19 ml. concentrated HCl to 1 liter with distilled water. Standardize by titrating against primary standard grade Na_2CO_3 (directions can be found in most general analytical texts).

i. pH indicators: Phenolphthalein—dissolve 1 g. in 70- to 90-percent ethanol; brom cresol green—thoroughly mix 0.1 g. brom cresol green with 14.3 ml. 0.01 *N* NaOH in a mortar (or in a beaker using a large stirring rod) and dilute to 100 ml. with distilled water.

2. Oxidation-diffusion procedure:

The semimicro method of oxidation is employed, including at least one reagent blank. However, all of the reactants cannot be combined before the reaction flasks are sealed. Therefore, the dichromate and acid are added directly into the flasks and the samples are placed in the sample cups which are inserted upright into the flasks. The following steps refer to each sample in a series.

a. If the sample contains inorganic carbon (carbonates or bicarbonates), moisten it with dilute sulfuric acid (5 percent) and allow several minutes for the CO_2 to diffuse out. CO_2 removal may be hastened by placing the acidified sample under vacuum. Samples contain-

ing large amounts of inorganic carbon require special pretreatment (Shaw, 1959).

b. With the reactants in place, insert an absorption tube containing 2 or 3 ml. of 2 *N* NaOH and stopper the flask (the hydroxide can be measured in the 10-ml. graduated cylinder). Draw a vacuum on the flask, and close the access clamp. Swirl or tilt the flask to overturn the sample cup, and continue swirling for about 30 seconds. If the absorption tube is of the proper length, there will be little danger of spilling the hydroxide.

c. Place the flask in a boiling water bath, following the oxidation procedure. The hydroxide may become desiccated toward the end of the reaction period, but apparently this does not affect CO_2 absorption.

d. Remove the flask from the bath, release the vacuum, and remove the absorption tube. The reaction solution adhering to the lower outer surface of the tube must be rinsed back into the flask. Stopper the tube if immediate analysis is inconvenient. Analyze the contents of the flask as in the oxidative method.

3. Carbon determination:

a. Rinse the contents of the absorption tube into a titration flask and dilute with CO_2-free water to about 20 ml.

b. Add 2 or 3 ml. of 2 *N* barium chloride (measured in the 10-ml. graduated cylinder) and swirl. A barium carbonate precipitate forms in proportion to the amount of CO_2 absorbed.

c. Add 1 drop of phenolphthalein and titrate with HCl until the color disappears. This acid volume is not recorded but the endpoint must be observed carefully.

d. Note burette reading, add 3 or 4 drops brom cresol green, and resume titrating to the yellow endpoint. The precipitate disappears as titration proceeds, and near the endpoint, color changes occur prematurely because of the free CO_2 in solution. When this occurs, expel the carbon dioxide by heating the flask to boiling. Cool it rapidly under running water, and resume titrating to the endpoint. Note the volume of acid used. The reagent blank will require a large volume of HCl to reach the phenolphthalein endpoint, but only a few drops in the final titration if reagents were properly prepared and contamination minimized.

4. Calculation of sample carbon content:

Mg. carbon = (sample titer − reagent blank titer) × *N* HCl × 6.

5. Example:

Two series of analyses of carbon in ammonium tartrate are given in table 5 with nitrogen determinations which will be described in the following section. Five ml. of 1 *N* dichromate and 10 ml. of acid were employed in oxidation. In one series, the tartrate was weighed and added in a dry state; in the other, dichromate was evaporated in the reaction flasks, and the tartrate was added as a solution of known concentration (5 ml. at 9.632 g/L.). The weights of carbon found have been converted to percentages to enable comparison of dissimilar samples. These analyses indicate an accuracy of 99 percent with a precision within ±2 percent of the mean value. Other easily oxidized compounds (e.g., oxalate, lactose, cellulose) have given similar results, but the method has not yet been used with resistant purified compounds. Limnological samples that have been analyzed by this method with only slightly lower precision are presented in part VI.

6. Comments:

The transformations of carbon in this procedure are summarized in the general reaction scheme:

organic carbon + O_2——(oxidation)→CO_2;

CO_2 + 2NaOH——(absorption)→Na_2CO_3;

TABLE 5.—*Analyses of carbon and nitrogen in ammonium tartrate*

Element analyzed and sample weight (mg.)	Oxygen Equivalent [1]	Carbon		Nitrogen (duplicate series)			
		Milligrams found	Percent of sample	Milligrams found		Percent of sample	
				A	B	A	B
Carbon:							
51.7 mg.	0.429	13.50	26.2				
40.9 mg.	.433	10.49	25.7				
45.7 mg.	.434	11.72	25.6				
Mean	.432		25.8				
Nitrogen:							
40.9 mg.	.440			6.00	6.00	14.7	14.7
43.5 mg.	.427			6.51	6.50	15.0	14.9
41.6 mg.	.434			6.00	6.26	14.4	15.0
Mean	.434					14.8	
Carbon and nitrogen:							
48.16 mg.	.435	12.34	25.6	7.03	7.32	14.6	15.2
48.16 mg.	.435	12.73	26.4	7.70	7.35	16.0	15.2
48.16 mg.	.435	12.37	25.7	7.35	7.52	15.2	15.6
Mean	.435		25.9			15.3	
Mean percent of sample			25.9			15.1	
Mean percent of theory [2]			99.0			98.8	
Average deviation—percent			±1.2			±2.3	
Coefficient of variation—percent			±1.3			±3.0	

[1] Milligrams oxygen per milligram sample.
[2] Theoretical values are given in table 10.

$Na_2CO_3 + BaCl_2$——(precipitation)$\rightarrow BaCO_3$;
$BaCO_3 + HCl$——(titration)$\rightarrow CO_2$.

Carbon dioxide can be titrated as sodium carbonate, but precipitation with barium offers two benefits: it permits a sharp phenolphthalein endpoint (at pH 8.0) and it retains the carbon in a divalent state (equivalent weight = 6), thereby requiring twice the acid volume needed to titrate carbon as HCO_3^{-1}.

Two interferences probably occur to some extent in this procedure when complex organic matter is analyzed. Sample chloride, oxidizing to Cl_2 is expected to diffuse into the absorption tube and react to form chloride and hypochlorite. The presence of the latter has been manifest on occasion by the fading of phenolphthalein color (oxidation). A more important interference may be caused by the absorption of acetic acid (see part III), most of which would be titrated at the brom cresol green endpoint. This titration would account for only one-fourth of the carbon present as acetate. Although the occurrence of acetate is known only from indirect evidence, the circumstance, if true, could prove advantageous. The presence of hypochlorite might be used to advantage also. These two compounds (acetate and oxidized chloride) represent the major constant error in oxidative efficiency. An analysis of them in the absorption solution, which seems quite possible, would permit an insight into the composition of the sample and an accurate correction for the oxidation results.

Carbon dioxide diffuses rapidly from the reaction solution into the absorption tube. It is formed, however, at a definite rate which depends mainly on the stability of the organic matter; therefore, when natural organic matter, lipid, or proteinaceous material is oxidized, the flasks should be left unopened for about an hour after they are removed from the water bath. Another factor to consider is the water of the absorbing solution, which diffuses slowly into the reaction mixture because of the desiccating property of the acid and thereby lowers the acid concentration. Since this may reduce oxidative efficiency, a maxi-

mum hydroxide volume of 3 ml. has been suggested. A precaution which can be exercised is to begin the oxidation with a slightly higher acid:water ratio (e.g., 2.2:1). Evacuation of the reaction flasks is not an essential feature of this procedure, but merely hastens the diffusion process. Good carbon recoveries have been obtained by using unevacuated, 125-ml., screwcap Erlenmeyer flasks, allowing them to remain sealed for a few hours after removal from the water bath.

This diffusion analysis apparently can be applied readily to samples of the size employed in the micro method of oxidation. The method of Katz et al. (1954) concerns samples containing about 1 to 5 mg. carbon. Kay (1954) described a related technique in which carbon is estimated in 10-ml. samples of sea water (about 0.03 mg. C) with a precision of ±3 percent. Many modifications of this method are possible, and the interested reader is referred to Conway (1958) for a background on diffusion principles and applications, and to Kolthoff and Sandell (1952) for considerations of reagents, indicators, and reactions.

Nitrogen analysis

It has been noted that organic nitrogen appears as an ammonium salt in the dichromate oxidation reaction. A method was needed to estimate this nitrogen which would not prevent normal oxidative analysis. Ammonium nitrogen is stable in acid solution, but volatilizes as ammonia when the solution is made alkaline, as in a Kjehldahl distillation. Micro Kjehldahl distillations were, in fact, tried on small portions of the reaction mixture with varying success, but this procedure lacked the versatility desired for compatibility with the preceding analyses. Micro diffusion possibilities were investigated and a suitable procedure was developed on the general pattern of the Conway (1958) methods. The diffusion is accomplished with small aliquots of the reacted oxidation mixture in separate vessels. The ammonia is absorbed in a boric acid solution and titrated with HCl. The following procedure specifically concerns sample nitrogen contents representative of limnological material normally used in the semimicro reaction (0.5 mg. N or more).

1. Apparatus and reagents:

Except for the omission of silver sulfate in the acid (see "Comments," below), oxidation requires the same glassware and reagents as the semimicro method, with or without concommitant carbon analysis. They should be as ammonia-free as possible. The following items refer exclusively to the nitrogen analysis.

a. Diffusion dishes: Conway "units" with internal absorption wells are standard, but any covered dishes having optimal dimensions (Conway, 1958) may suffice. Stender dishes, 5 cm. in diameter with silicone grease in the lid grooves, were used here. Polyethylene vial caps, 25 mm. wide and 15 mm. deep, were satisfactory absorption cups.

b. Volumetric glassware: One 50-ml. volumetric flask for each sample and reagent blank in a series, a 1-ml. and a 3-ml. pipette, and 2-ml. Koch-type microburette are recommended. Larger burettes are usable with less concentrated acid.

c. Ammonia-free distilled water: Redistill from acidified solution, perfuse through an appropriate exchange resin, or shake with permutit (see A.P.H.A., 1955).

d. Mixed indicator, 0.1-percent alcoholic solution: Dissolve 33 mg. brom cresol green and 67 mg. methyl red in a few ml. 95-percent ethanol and dilute to 100 ml. with ethanol.

e. Boric acid absorbent, 1-percent solution with mixed indicator: Dissolve 10 g. H_3BO_3 in 700 ml. distilled water and 200 ml. 95-percent ethanol. Add 10

ml. indicator solution (above), mix, and make up to 1 liter. Adjust, if necessary, to a weak reddish tint (pH about 5) with a few drops of dilute NaOH.

f. Standard HCl, 0.01 *N*: prepare by diluting the standard acid described for carbon analysis with distilled water. The exact normality is calculated from the dilution ratio and determined normality of the stock HCl.

g. Potassium hydroxide, pellets: Fresh stock, low in carbonate.

2. Oxidation procedure:

The oxidation procedure of the semimicro method, with or without a carbon determination, is followed explicitly. It is assumed that the sample contains no reduced inorganic nitrogen (ammonium *N*). If such nitrogen is present, the sample should be treated with a small amount of dilute hydroxide and the liberated ammonia allowed to diffuse out before proceeding with the oxidation. After the oxidation is completed, the reacted mixture is rinsed into a 50-ml. volumetric flask with enough ammonia-free water to make a total diluted volume of about 49 ml. This solution is mixed well, allowed to cool, and made up to 50 ml. At least half of it (25 ml. or more) is used for the Oxygen Consumed determination. The remainder can be stored tightly stoppered if immediate nitrogen analysis is inconvenient.

3. Nitrogen diffusion and titration:

A precisely measured amount, from 1 to 5 ml., of the oxidized sample solution is required for each nitrogen determination. Any amount taken below 5 ml. must be diluted with ammonia-free water to this volume, which is optimum for the dish size and neutralization conditions specified here. The following operations refer to an "average" aliquot of 3 ml. Effective neutralization of this solution requires manipulative proficiency which is easily attained with a little experience. Neutralization must be complete in every respect and the only acid present in the diffusion dish after that time should be within the absorption cup. As in previous procedures, it is expedient to analyze a series of samples simultaneously, reagent blank included. A single member of such a series is described below.

a. Pipette a 3-ml. aliquot of the sample solution into a clean diffusion dish, add 2 ml. ammonia-free water (it can be measured in a small graduated cylinder), and swirl the dish gently to mix the contents.

b. Count out the number of KOH pellets needed to neutralize this solution, and include one additional pellet. The necessary amount of hydroxide can be estimated from the supposed acid content of the sample aliquot. In this case, it will be about 1.2 g. or 6 to 8 pellets. Assume that 7 pellets, including the extra one, are required. Add 3 of them to the dish while gently swirling it and continue to swirl until the pellets have dissolved. At no time should the pellets be exposed above the surface or allowed to effervesce, else a premature evolution of ammonia will occur. Add 2 more pellets and repeat the above operation. When these have dissolved, the solution should still be acid and light amber in color (cf., reaction of dichromate with hydroxide in part III). The pH can be tested by touching the tip of an indicator paper to the surface of the solution. Cover the dish lightly and set it aside.

c. Pipette 1 ml. of boric acid into the absorption cup. Precise measurement is unnecessary, but all members of the series should contain about the same amount. If the nitrogen content of the aliquot is more than 0.2 mg., use 1.5 ml. absorbent.

d. Remove the lid from the diffusion dish, place the absorption cup inside, and with the lid in readiness place the remaining 2 KOH pellets in the partly neutralized solution. Quickly press the

lid into place, and tilt the dish with a precessing motion to dissolve the pellets (the sample solution should not, of course, contact the absorbing solution). The sample solution should now be alkaline. This fact will be evidenced by a white precipitate which forms during the final neutralization and by the color change of the chromium compounds (see part III). Sample solutions will usually turn light green, and the reagent blank, a light yellow. Another indication of proper pH change is the color change of the absorbent, which becomes green after a few minutes as a result of ammonia absorption. Heat evolved in the final neutralization may cause temporary internal pressure, making it advisable to clamp or weight the lid until the dish has cooled.

e. Set the dish aside for a few hours (preferably overnight) until ammonia diffusion is complete. An occasional rotation of the dish during this period will hasten diffusion.

f. Remove the absorption cup, and titrate its contents in situ to a red endpoint with standard HCl. Various but distinct shades of red are possible. If a reagent blank is titrated first (it should not require more than a drop or two of acid), the samples can be titrated to the same color with ease.

4. Calculation of organic nitrogen content:

The nitrogen content of the aliquot analyzed is given by the equation

$$\text{mg. N} = (\text{sample titer} - \text{reagent blank titer}) \times N\ \text{HCl} \times 14,$$

and the nitrogen content of the original sample will be

$$\text{sample N, mg.} = \frac{\text{mg. N in aliquot} \times 50}{\text{ml. aliquot}}.$$

5. Example:

Nitrogen recoveries from two ammonium tartrate oxidation series are reported in table 5, one of them done in conjunction with organic carbon determination. Duplicate aliquots were analyzed for each sample, and the nitrogen quantities found were converted to percentages of sample weight for comparative purposes. The accuracy of the method on this compound is 99 percent of the theoretical value, with a precision of 3 percent (coefficient of variation). The accuracy and precision with other pure compounds have not yet been tested, but favorable results were obtained with limnological samples, as described in part VI.

6. Comments:

Some advantages of this method are: it is adaptable to a large number of concurrent analyses; it allows normal oxidative analysis, even when replicate aliquots of the sample solution are taken; only one standard solution, one exact pipetting, and one titration are required for each determination; diffusion units are simple and may be fashioned from various items of laboratory glassware. The chief disadvantages are the time required for diffusion and the care needed to avoid contamination when working with low levels of ammonia N. The diffusion period, however, can be devoted to other activities, since the process requires no attention once the units have been prepared.

A black precipitate was observed to form in the neutralized solution when the oxidation included catalytic silver. Silver sulfate was omitted thereafter, since the effect of this precipitate on ammonia diffusion is unknown. The absence of silver is expected to lower the completeness of oxidation of resistant compounds by a few percentage points (see part III). This reduced efficiency primarily affects carbon recoveries (cf., pages 9 and 13). All organic N, imino N possibly excepted, should be converted to ammonium salts if the sample is thoroughly exposed to the oxidizing mixture.

Quite probably, the silver precipitate does not interfere with nitrogen analysis—a premise which will be tested in future work.

Both the precision and the accuracy of the method reside mainly in the diffusion process. The rate of ammonia transfer, from the sample solution into the absorbent, is controlled principally by its diffusion rate within the former solution. The surface area of this solution with respect to its depth becomes a limiting factor which is affected directly by the dimensions of the diffusion vessel. Diffusion rates will also vary with the neutralizing agent used, but are not dependent upon the final alkalinity attained (so long as ammonium is converted to ammonia). These factors are discussed at length by Conway (1958). For any given diffusion vessel, the smallest volume of sample solution which can cover the bottom area will be the most efficient. Strong acidity of this solution in the present procedure imposes limits on the volume employed and the type of neutralizing agent which can be used effectively.

Concluding remarks

These methods of organic carbon and nitrogen analysis have not been investigated as thoroughly as the oxidation technique. They conform well with the objectives of method simplicity and versatility which are desired, and appear to be useful limnologically in their present state of development. If the methods are employed in this capacity, it is recommended that reagent grade ammonium tartrate be included in occasional analyses as a standard substrate. Further testing is expected to result in improved procedures and in a better knowledge of the character and reliability of the results. Data given by these methods can be combined with oxidation values to provide additional organic characterizations which are described in the following text.

Part V. SIGNIFICANCE AND UTILIZATION OF ANALYTICAL DATA

Oxidation values obtained with the foregoing procedures are applicable to the estimation of organic mass, energy, and composition. The requisite conversion factors, however, must be derived from considerations of the relation of oxidation oxygen to organic matter. In the following treatment, emphasis is placed on the characteristics of organic matter from natural environments because data from laboratory cultures are biased by unnatural conditions. Several concepts require supporting information from an interpretation of natural organic matter according to prominent compounds and proximate groupings. This interpretation proves too voluminous for direct inclusion in the text, and has therefore been deferred to the appendix.

TRANSFORMATIONS OF OXYGEN CONSUMED VALUES

The transformation of an Oxygen Consumed datum into more cogent organic values requires numerical conversion factors which express the desired relations. Since organic matter in any limnological sample is a broad assemblage of various compounds, these factors must necessarily be averages which carry certain assumptive risks. An attempt has been made to minimize errors of assumption by an extensive consideration of the relevant characteristics of natural organic matter. Three conversions of the O.C. value described below (to total organic matter, organic carbon, and caloric content) are direct, 1-step transformations, and a fourth employs both O.C. and elemental composi-

tion data in estimating total organic matter from calculated proximate components.

Direct estimation of total organic matter

The factor which converts the Oxygen Consumed by a sample to organic weight is the theoretical Oxygen Equivalent of the organic matter it contains. For instance, the milligrams of oxygen reacting with any amount of a pure organic compound, when divided by its O.E. (mg. oxygen per mg. compound), will give the weight of the compound that was oxidized. To convert the O.C. values of natural samples to organic weights, it is necessary to assume a single O.E. that is universally representative. Some proposed conversion factors have been based on the O.E. of a single nonrepresentative compound, such as a hexose sugar (O.E. = 1.06). Vinberg (1954) has employed the relation 1 mg. oxygen = 0.75 mg. ashless plankton (or O.E. = 1.33) in the study of lake seston.

A universal factor may be determined empirically from the oxidative analyses of samples whose organic content is known with a reasonable degree of certainty. An example is given in table 6, which lists both the O.C. values (as weight ratios) and the experimental O.E.'s. of seven limnological substrates. The former values are included to show the variability which occurs among them as a result of different ash and residual moisture contents. The O.E.'s. fall into a rather narrow range (1.38 to 1.46) with a median of 1.42 mg. O/mg. organic matter. These few samples, however, do not provide an unequivocal basis for assuming a universal conversion factor. Prominent sample types missing are seston, sediments, and total dissolved organic matter. Many limnological materials are not available in sufficient quantity or are proportionately too low in organic content to permit this sort of interpretation. It is necessary, therefore, to use other methods to determine a representative O.E.

Another way in which the Oxygen Equivalent can be determined for a mixture of unknown organic compounds, such as a natural sample, was described in part III. The O.E. is calculated from a hypothetical molecule whose atomic ratio is derived from the ultimate composition of the mixture. A few relevant ultimate analyses found in limnological literature have been recalculated as indicated, and their data are presented in table 7. These O.E.'s are more variable than the ones above, ranging from 1.20 to 1.71 mg. O/mg. sample. The number and classes of samples also are too few to give a representative "average" value.

A final approach to the determination of a universal conversion factor (O.C. to organic matter) is made through an evaluation of proximate analyses selected from several literature sources. This method of assigning O.E.'s. to

TABLE 6.—*Experimental Oxygen Equivalents and related analytical factors for some natural organic substrates*

Factor	*Aphanizomenon*	*Batrachospermum*	*Cladophora*	*Daphnia*	*Gloeotrichia*	Humic extract	Neuston
Oxygen Consumed (air-dry basis)	1.22	1.09	1.12	1.04	1.21	0.67	1.18
Moisture + ash percent	8.9	26.8	28.1	25.1	9.8	51.1	13.2
Oxygen Equivalent [1]	1.41	1.38	1.44	1.46	1.41	1.44	1.43
Source [2]	A	B	B	A	A	C	D

[1] Calculated from Oxygen Consumed values by adjusting them to a moisture + ash–free basis and allowing for 5 percent incomplete oxidation.

[2] Sources of substrates: A are plankton organisms from Crowley Lake, Calif.; B are attached algae from Beaver Brook, Cortland County, N. Y.; C is a precipitate (by $CuSO_4$) from acid lake water taken in Adirondack State Park, N. Y.; D is a surface film (microorganisms) obtained from a pond in Cortland County, N. Y.

TABLE 7.—*Oxygen Equivalents of some limnological substrates calculated from their reported elemental compositions*

Substrate	Elemental atomic ratios				Valence change [1]	Oxygen Equivalent
	Carbon	Hydrogen	Oxygen	Nitrogen		
Copepods [2]	4.43	8.13	1.67	0.87	19.90	1.59
Peridineans [2]	4.02	7.39	2.12	0.75	16.98	1.36
Diatoms [2]	4.20	10.10	1.80	0.74	21.40	1.71
Humic precipitate [3]	4.10	4.46	2.74	0.17	14.97	1.20
Humolimnic acid [4]	4.53	5.59	2.44	0.09	18.56	1.48
Crenic acid [5]	4.14	8.22	2.48	0.16	19.34	1.55

[1] Valence change in oxidation per hypothetical molecule, molecular weight = 100.
[2] Brandt and Raben (1920). O.E. range for all diatom analyses reported was 1.60 to 2.01.
[3] Åberg and Rodhe (1942).
[4] Shapiro (1957). Acetate extract of lake water.
[5] Ponomareva and Ettinger (1953). Electrodialyzate of Neva River water.

samples is actually a 3-step process based on the premise that a group of chemically related compounds can be represented fairly accurately by a single O.E. The first two steps (i.e., the arrangement of limnologically important compounds into related proximate groups, and the characterization of each

TABLE 8.—*Oxygen Equivalents calculated for various limnological substrates from their reported proximate compositions*

Reference and substrate	Sample code [1]	Number of analyses	Percent			Oxygen Equivalent [2]
			Crude protein	Lipid	Crude carbohydrate	
Birge and Juday (1934):						
Raw lake water, carbon per liter:						
1–1.9 mg.	D	(data from 529 lakes)	26.4	2.4	71.2	1.46
5–5.9 mg.	D	do	21.4	1.6	77.0	1.44
10–10.9 mg.	D	do	16.9	0.7	82.4	1.43
15–15.9 mg.	D	do	14.5	0.5	85.0	1.42
20–25.8 mg.	D	do	12.3	0.3	87.4	1.42
Weighted mean			17.2	0.9	81.9	1.43
Seston	Sn	529 lakes	37	4	59	1.39
Organic sediments	St	66	26.5	2.6	70.9	1.46
Dissolved organic matter:						
Diamond Lake, 3.26 mg. per liter	D	unknown	28.5	3.3	68.2	1.48
Helmet Lake, 55.3 mg. per liter	D	unknown	8.1	0.6	91.3	1.42
Birge and Juday (1922):						
Net plankton:						
Mendota Lake	N	84	51.2	12.2	35.7	1.55
Monona Lake	N	21	62.8	7.0	30.2	1.50
Waubesa Lake	N	16	49.5	4.0	45.5	1.40
Nannoplankton:						
Mendota Lake	Sn	87	42.8	6.6	50.6	1.43
Monona Lake	Sn	21	55.8	4.8	39.4	1.44
Waubesa Lake	Sn	16	46.8	5.8	47.4	1.43
Organism groups:						
Cyanophyta (5 genera)	P	10	57.2	3.2	39.6	1.45
Volvox	P	1	50.8	5.9	43.3	1.47
Diatoms	U	1	37.8	22.5	39.7	1.65
Copepoda (2 genera)	I	4	63.2	16.5	20.3	1.62
Cladocera (3 genera)	I	14	59.3	11.8	28.9	1.54
Aquatic insecta (5 orders)	I	7	62.0	12.0	26.0	1.55
Geng (1925): Aquatic invertebrates:						
Insecta (5 orders)	I	7	63.0	11.4	25.6	1.54
Crustacea (3 orders)	I	5	71.1	8.1	20.8	1.51
Mollusca (3 genera)	I	4	54.3	7.4	38.3	1.48
Hsiao (1949):						
Zooplankton	I	3	65.9	19.3	14.8	1.69
Phytoplankton	P	7	45.0	7.3	47.7	1.40
Krogh and Lange (1932): Lake Furesø samples:						
Plankton	N	unknown	53.2	9.9	36.9	1.52
Colloidal matter	U	12	41.5	11.7	46.8	1.60
Dissolved matter	D	15	37.5		62.5	1.44
Krogh and Berg (1931): Schlosssee plankton	N	18	53.6	10.7	35.7	1.53
Matida and Sakurai (1953): River periphyton	P	2	21	9	70	1.40

[1] Samples are coded by the type of organic matter they contain: D = dissolved organic matter; I= aquatic invertebrates; N = net plankton (probably mainly zooplankton); P = phytoplankton (and other plant cellular material); Sn = seston; St = organic sediments; U = unclassified.
[2] Factors assumed for proximate groups in calculating total Oxygen Equivalent: $O.E._P = 1.5$; $O.E._L = 2.7$; $O.E._C = 1.2$ (except D and St samples where $O.E._C = 1.4$).

group by its representative O.E.) have been accomplished in the appendix. The last step is indicated in table 8, which lists various proximate analyses that have been recalculated to ash-free unit values where necessary. A sample O.E. is found by summing the products of the proximate component proportions times the respective O.E. constants.

This procedure is less reliable for a single sample than the ones described previously because it assumes true representation by the O.E. constants, and accuracy of the proximate analyses. The resulting error is expected to decrease as individual analyses are combined, and therefore greater emphasis should be placed on the O.E.'s. calculated from the data which are averages of many analyses. The abundance of data in table 8 permits considerations of sample types, but such considerations can be made only in general terms because of many minor confounding differences among the various analyses. The following is an interpretation of the characteristic O.E.'s. of the different sample types noted in table 8 (sample types are listed in the approximate order of decreasing limnological importance):

Sample type	*Table code*	*O.E. range*	*Representative O.E.*
Dissolved matter	D	1.42–1.47	1.44
Seston	Sn	1.39–1.53	1.40
Organic sediment	St		1.46
Phytoplankton	P	1.40–1.47	1.44
Aquatic invertebrates	I	1.48–1.69	1.54
Net plankton	N	1.40–1.55	1.52

It is not possible to classify the datum on colloidal organic matter into this scheme. The single diatom datum was omitted because diatoms are known to contain large amounts of lipid under certain conditions and in this respect are similar to plankton crustaceans (see table 7). The direct relation between lipid content and O.E. is strongly indicated in table 8.

The three methods of determining sample O.E.'s. give results that are in general agreement. The most representative values appear to be in the range 1.4 — 1.5 mg. O/mg. sample organic matter, although high-lipid substrates have O.E.'s. that are definitely higher. The types of samples most concerned with the conversion factor being determined are dissolved organic matter, seston, and sedimented matter. The O.E. characteristic of these samples, 1.43, is selected as a "universal" factor for converting O.C. values to organic weight. This may be accomplished by division, but it is more convenient to multiply an O.C. by the reciprocal, 1/1.43, which may be rounded to 0.7. The relative error involved in using this factor for comparable samples will be small. The approximate absolute error can be shown by considering "true" O.E.'s. at the extremes of the normal range. Assume that two different samples have actual O.E.'s. of 1.3 and 1.7. If their O.C. values (observed) are converted to organic weight by the suggested factor (1.43) the estimate will be 9 percent too low in the first case and 19 percent too high in the second. Generally, however, calculated organic weight is expected to be within ±10 percent of the true value, assuming perfect analytical accuracy.

Estimation of organic carbon

Although the analytical methods described here include the direct determination of organic carbon, it may be desirable, instead, to estimate the sample organic carbon content from the analytical Oxygen Consumed. This conversion is actually less subject to assumptive error than the conversion to organic matter because the O.C. varies with carbon content more closely than it does with organic mass.[2] The factor that

[2] Referring to the values obtained in the appendix, 1 gram of oxygen will oxidize 0.83 gram of crude carbohydrate containing 0.38 gram of carbon, but will oxidize only 0.37 gram of lipid containing 0.28 gram of carbon.

transforms O.C. to carbon is the Oxygen Equivalent of carbon, which is the O.E. of a compound divided by its fractional carbon content. In part III, the carbon O.E. is indicated as the effective valence change of carbon in a molecular oxidation, times 8 (to get oxygen weight), divided by the weight of carbon in the molecule. The O.E. factor needed for the conversion of any observed O.C. to organic carbon, as for the conversion to organic matter, is a single value which is representative of the effective valence state of organic carbon in limnological samples.

The conversion to organic carbon is frequently given in the literature on the basis of a carbon valence change of 4 electrons (see part III) i.e., that elemental carbon or true carbohydrate is being oxidized. The transformation described by El Wakeel and Riley (1957) serves as an example (it was adapted from Allison, 1935):

$$\text{ml. } 0.2N\ Fe^{+2} \times 0.6 \times 1.15 = \text{mg. carbon.}$$

The first term of the equation is the net titer, each milliliter being the equivalent of 1.6 mg. of oxygen. The second term indicates that 0.6 mg. of carbon are equivalent to the first term (or that 32 mg. of oxygen are equivalent to 1 mg. atomic weight of carbon). The third term, 1.15, is stated to be a factor which corrects the calculated carbon to the true weight as determined by dry combustion analysis. It is, in fact, a factor which corrects partly for incomplete oxidation and partly for the inappropriate value of the second term (according to the valence interpretation discussed here in part III).

An appropriate conversion factor can be found by treating natural organic matter in a manner similar to that employed above in finding an O.E. representative of limnological substrates. This approach is considered unnecessarily meticulous because the carbon can be determined directly, if necessary. It is obtainable more easily by considering the average amount of carbon in natural organic matter and then properly adjusting the "universal" O.E. to a carbon O.E. Waksman and Hutchings (1935) discussed the carbon contents of various natural organic materials and indicated a carbon range of 50 percent to 58 percent in the organic matter of plant residues, humuses, peats, and composts. The data of Birge and Juday (1934) show carbon percentages of 46 to 48 in dissolved and sestonic organic matter. Of 17 plankton analyses presented by Brandt and Raben (1920), 15 place carbon between 47 percent and 53 percent of sample organic weight. Skopintsev (1948) stated that the carbon content of organic matter in river waters near Moscow is usually near 50 percent. The consensus seems to be that natural organic matter is very nearly 50 percent carbon. The representative carbon O.E. is therefore 1.43×2 (or 2.86). Its reciprocal (1/2.86 or 0.35) is a multiplication factor for converting O.C. to organic carbon.

The error of assumption in this conversion can be roughly described by assuming a certain range for the actual carbon content of the sample organic matter. If it is allowed that most limnological organic matter contains 46 to 54 percent carbon, the calculated carbon content for any sample will be within ± 8 percent of the true value if the O.C. and assumed O.E. are accurate. It is possible to describe this error relative to the error in calculating organic content by a consideration of the theoretical O.C. and carbon content of a hypothetical sample, and values calculated therefrom. If this is done, it will be apparent that the calculation error for carbon is only half that of the organic matter error. In most circumstances where the oxidation is accurate, the calculated car-

bon content of a sample should be within ±5 percent of the true value.

Estimation of proximate composition

It is possible to calculate the proximate composition of sample organic matter in terms of weights from the analytical values for O.C. carbon, and nitrogen. The method, however, involves several independent assumptions and is less reliable than a different method described in the following section. For this reason, only a brief outline of the calculation will be given here.

The foregoing conversions and the considerations in the appendix point out the relations that exist between proximate groups, their carbon content, and their assumed Oxygen Equivalents. These relations can be combined in two parallel equations which are solved by inserting three analytical values. The values for O.C. and weight of carbon are employed directly, while the organic nitrogen is converted first to crude protein. The latter derived value is used, in turn, to calculate the weight of protein carbon, which is the third value needed. The equations are:

$$(1) \quad C_C + C_P + C_L = C_T$$

$$(2) \quad 2.66\,C_C + 2.89\,C_P + 3.86\,C_L = \text{O.C.},$$

where C_C, C_P, and C_L are the weights of carbon in crude carbohydrate, crude protein, and lipid, respectively; C_T is the total organic carbon in the sample; O.C. is the sample Oxygen Consumed.

The numerical constants 2.66, 2.89, and 3.86 are carbon O.E.'s for the respective proximate groups (K = group O.E. × 100/percent carbon) and serve to convert carbon weights to oxygen weights. Analytical values for C_T and O.C., in the same weight units, are inserted directly. C_P is calculated for the sample:

$$\text{Weight protein carbon} = \frac{\text{weight organic N} \times 6.25 \times 100}{52}$$

and is inserted in both equations. Equation 1 is then multiplied through by 2.66 and subtracted from equation 2, giving a final equation containing one unknown, C_L, which is easily calculated. C_C is the difference, $C_T - (C_P + C_L)$. The weight of the proximate groups is obtained from their assumed carbon percentages:

$$\text{Weight crude carbohydrate} = C_C \times 100/45,$$

$$\text{Weight lipid} = C_L \times 100/70.$$

Crude Protein weight has already been determined (weight organic N × 6.25). Finally, the weight of the proximate groups can be summed to give an estimate of the total weight of organic matter in the sample.

Estimation of organic energy

The potential energy of organic matter in biosystems is related more to the state of reduction than to the organic mass, and is nearly always measured as heat (calories) by combustion calorimetry. These general associations have been discussed by West (1956), who also pointed out that the total energy given off by a substrate in oxidation is independent of the pathway as long as the end products are identical. While the temperature changes observed in combustion calorimetry provide a standard method of measuring organic energy, the relation between this energy and Oxygen Consumed is close enough to permit an accurate caloric estimate by quantitative oxidation. The importance of this relation was stressed by Spoehr and Milner (1949). Oxidative analyses have been proposed for organic energy characterization by Ivlev (1934) and Krogh (1930), and applied in the studies of Krogh and Berg (1931), Krogh and Lange (1932), and Vinberg et al. (1934). The transformation of an Oxygen Consumed datum to caloric value

involves less assumptive risk than the previously described conversions, and requires a conversion factor representative of limnological organic matter.

The energy liberated in organic oxidation was described by Kharasch (1929) as the result of a rearrangement of interatomic bonds from higher to lower energy states. Different types of bonds represent different energy levels. Kharasch has taken the bond energy of methane oxidation in terms of valence change as a base value (26.05 kilocalories per electron mole) to demonstrate a method for calculating heats of combustion for pure compounds with an accuracy of about 1 percent. The method involves multiplying electron moles required for oxidation (valence change) by 26.05 and adjusting the product to a final value with certain energy factors attributable to specific configurations. Earlier, Thornton (1917) had deduced a combustion caloric value of 53 kilocalories per gram atom of oxygen by comparing observed heats of combustion with the amounts of oxygen required. Since 1 gram atom of oxygen equals 2 electron moles, the two conversion bases are in close agreement. Kharasch's adjustments are mostly addition factors, so the relation 26.5 kilocalories per electron mole (or 3.31 kcal./g. oxygen) can be taken as representative.

The conversion factors of both Kharasch and Thornton are based primarily on hydrocarbons which have less energy per unit weight of Oxygen Consumed than many biologically eminent compounds. The differences are not large, but a more representative factor is obtained by a consideration of the caloric and oxidation values of important limnological compounds. This has been done in the appendix by dividing the available standard thermodynamic heats of combustion (as kcal./g.) by the theoretical O.E.'s. of the respective substrates. Rounded values assumed for coefficients of the proximate groups from these data (as kcal./g. oxygen) are: Crude carbohydrate, 3.5; crude protein, 3.3; lipid, 3.3. The single representative value selected is 3.4 (or 27.2 kcal./ electron mole) which agrees closely with the factor 3.38 suggested by Ivlev (1934) and Vinberg et al. (1934). More often gram calories are calculated because the analytical oxygen datum is expressed in milligrams. The equation is: Sample gcal. = O.C. in mg. $\times$ 3.4.

The total calories found in this manner for nitrogen-containing samples do not compare directly with bomb calorimeter results because the latter assumes the oxidation of organic nitrogen to N_2. In most cases, biologically available energy does not concern nitrogen oxidation calories, and the decision as to which expression is most appropriate rests with the investigator. Calorimeter and oxidation data for nitrogenous materials can be adjusted to mutual agreement by appropriate addition or subtraction of nitrogen calories obtained from the relation N kcal. = g. organic N $\times$ 5.5. This relation is not exact because organic nitrogen bonds exist at various energy levels that cannot be evaluated easily.

Ivlev (1934) has calculated oxycalorie coefficients for 16 invertebrates (analyses reported from Geng, 1925, and Brandt and Raben, 1920) and found them to vary between 3.33 and 3.49. His own data indicated an accuracy of oxidation calorie determinations within ± 5 percent of bomb calorimetry values. The error associated with the assumed conversion factor is not very large. If the true oxycalorie coefficients for natural samples vary between the extremes indicated for cellulose (3.53) and palmitic acid (3.26), the calculated calories will be within ± 4 percent of the actual calories.

Since the Oxygen Consumed value of a sample more accurately represents organic energy, its conversion to calories is preferred to the other transformations described. The ratio of calories to carbon weight (determined, not calculated) can be useful in characterizing the organic energy level of a sample whose content of total organic matter is unknown. Fat, for instance, is distinguished by its high energy content per unit weight (about 9.4 gcal./mg.), which can be expressed in terms of its carbon content: 12.6 gcal./mg. carbon. Different compounds and also different samples have characteristic calorie/carbon ratios ranging up to 17.6 (methane).

UTILIZATION OF OXYGEN EQUIVALENT VALUES

An oxidative analysis is valuable even if the organic content of a sample is known. In this instance, the reaction serves as the first step in carbon and nitrogen analysis, and the quantity of oxygen which reacts provides a measure of the energy content of the sample. No less important is the experimental Oxygen Equivalent, which is used with the organic weight and the measured quantities of carbon and nitrogen to determine the proximate and ultimate compositions of the sample.

Determination of proximate composition

The nature of proximate groups with respect to limnological matter is treated in the appendix. In theory, these groups represent pure or closely related compounds. In analysis, however, total organic matter is merely partitioned by the methods employed into three groups which then contain some compounds that can be quite unrelated. Obviously, no two radically different methods will give identical results, nor can either be adjudged as more or less accurate so long as their results represent the defined or implied categories. The following method attempts to evaluate proximate groups analogous to the most common determinations: Crude protein is, by definition, organic nitrogen times 6.25; lipid conforms to organic extraction analysis, with a divergence described in the example; crude carbohydrate is the residual which brings the total to sample unity.

At least two oxidative methods of proximate group analysis have been described for limnological samples. Krogh (1930) used a dry combustion technique with organic carbon, nitrogen, and Oxygen Consumed measured by gasometry. These values were interpreted by respiratory quotients to give proximate groups. Spoehr and Milner (1949) derived proximate groups from ultimate components, also analyzed by dry combustion. Their calculations are similar to those given below, but use the "R-value" noted in part III. Their method has been used to estimate the proximate composition of cultured algae (Spoehr and Milner, 1949; Milner, 1948; Ketchum and Redfield, 1949).

The calculation of sample proximate composition requires two analytical values (in addition to the weight of organic matter), the O.E. and the organic nitrogen content, and three constants (proximate group O.E.'s.) taken from the appendix. Proximate percentages can then be derived from the parallel equations:

$$(1) \quad \%CC + \%CP + \%L = 100$$

$$(2) \quad 1.2\,\%CC + 1.5\,\%CP + 2.7\,\%L = O.E. \times 100,$$

where CC, CP, and L are crude carbohydrate, crude protein, and lipid, respectively. The quantity of nitrogen determined is converted to its percentage of organic matter and multiplied by 6.25 to obtain %CP. The latter is inserted into both equations. Equation (1) is

then multiplied through by 1.2 and subtracted from equation (2), which cancels out the first term. The result is factored and rearranged:

$$(3)\quad 1.5\ \%L = 100(O.E. - 1.2) - 0.3\ \%CP,$$

which is solved to obtain the lipid percentage. The crude carbohydrate percentage is then found by difference: $\%CC = 100 - (\%CP + \%L)$.

These determinations are frequently carried out on a large number of samples and the calculations may then become tedious. This condition can be averted by using a nomogram as shown in figure 4, which was prepared from equation 3 according to the method described by Levens (1948).

This oxidative determination of proximate composition is demonstrated in table 9 and compared with the results of "standard" analyses (crude protein from micro Kjehldahl nitrogen and lipid by ether extraction). The samples, trout fingerling and developing trout eggs, were the subjects of a physiological study reported by Phillips et al. (1959), and the "standard" compositions were taken from the original analytical data of that study. "Standard" protein values were used in the oxidative determinations because crude protein is synonymous in the two methods. Since both methods yield crude carbohydrate by difference, the comparison is more directly that of the lipid values.

A good agreement exists between the two methods for the percentages of lipid and crude carbohydrate found in trout fingerling. However, a striking difference is manifest in these values for trout eggs; the percentage lipid determined by oxidation is 2 to 3 times higher than by ether extraction. This anomaly is attributable to the nature of trout egg lipid, which Smith (1952) described as being distributed about equally between triglycerides (nonpolar) and phospha-

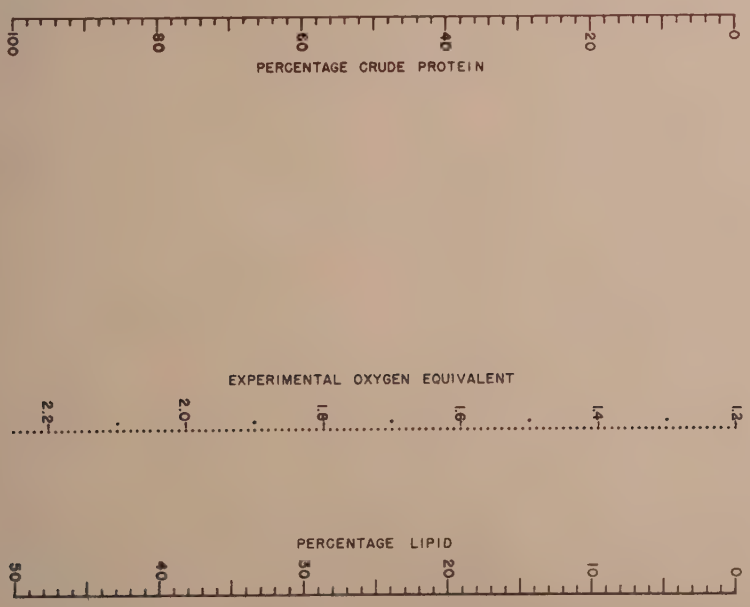

FIGURE 4.—Nomogram for the determination of lipid percentages from crude protein percentages and experimental Oxygen Equivalents. Lipid percentage is obtained by extrapolating a line connecting the two points representing the empirical values.

TABLE 9.—*Comparison of proximate compositions of trout samples determined by oxidative and "standard" analytical methods*

Sample Substrate [1]	Oxygen Equivalent	Percentage compositions [2]				
		"Standard" analysis			Oxidative analyses	
		Crude protein	Lipid	Crude carbohydrate	Lipid	Crude carbohydrate
Fingerling:						
Lot 1	1.69	60	22	18	21	19
Lot 2	1.80	48	33	19	30	22
Lot 3	1.64	59	19	22	17	24
Lot 4	1.81	47	30	23	32	21
Eggs:						
Lot 21	1.75	71	9	20	23	6
Lot 22	1.75	66	8	26	24	10
Lot 44	1.75	64	13	23	24	12
Lot 17	1.76	67	11	22	24	9

[1] Values for trout fingerling are means of 4 or more determinations; values for trout eggs are means of 2 or more determinations.

[2] "Standard" analysis by micro Kjehldahl and continuous ether extraction (Phillips et al., 1959); quantitative oxidation by the semimicro method. The same protein values apply to both analyses.

tides (polar). Smith cited data from several sources which indicate that total trout egg lipid is usually more than 20 percent of the total dry weight. He also suggested that relatively nonpolar solvents (ether, carbon tetrachloride) effectively extract only triglyceride fat. The oxidative determination is not founded on solubilities and will therefore give lipid values comprising all compounds distinguished by their strongly reduced state, or high energy content.

The equations and nomogram given here are based on one set of proximate group O.E.'s. which are assumed to be generally representative. Certain samples whose molecular composition differs strongly may be better characterized by substituting more appropriate constants (see appendix).

Determination of ultimate composition

The percentages of any two organic elements in a sample can be calculated from its empirical O.E. by parallel equations if the content of the remaining organic elements is known. The procedure has been described by Kuhn and L'Orsa (1931) and by Williams (1937). The four major organic elements, C, H, O, and N are of greatest concern, and the method will be explained for them. Carbon and nitrogen are determined analytically as previously described, and their percentages in the sample are used with the sample O.E. to calculate the hydrogen and oxygen percentages. The equations and procedure are similar to those used in proximate group determination:

$$(1) \quad \%C + \%H + \%N + \%O = 100$$

$$(2) \quad 2.7\ \%C + 7.9\ \%H - 0.7\ \%N - 1.0\ \%O = O.E. \times 100.$$

Letters designate the elements concerned. The numerical coefficients are true constants which have been calculated from the relation

$$\text{constant} = \frac{\text{initial valence} \times \text{equivalent weight oxygen}}{\text{atomic weight}}$$

and carry the sign of their valence number (C^{+4}, H^{+1}, N^{-3}, O^{-2}). The O.E. is the sample Oxygen Equivalent.

The hydrogen percentage is calculated by inserting the three empirical values (%C, %N, O.E.) into both equations 1 and 2. The equations are then added to cancel out the oxygen terms, and the result is factored and rearranged:

$$(3) \quad 8.9\ \%H = 100\ (O.E. + 1) + 0.7\ \%N - 3.7\ \%C.$$

TABLE 10.—*The ultimate composition of ammonium tartrate as determined by oxidative analysis*

Element	Percentage composition			Related data
	Experimental		Theoretical	
	Analyzed	Calculated		
Carbon	25.9		26.1	Formula: $C_4H_{12}N_2O_6$.
Nitrogen	15.1		15.2	Molecular weight: 184.15.
Hydrogen		6.6	6.6	Theoretical Oxygen Equivalent: 0.434.
Oxygen		52.4	52.1	Experimental Oxygen Equivalent [1]: 0.434.

[1] From table 5.

Equation 3 is solved for %H, and %O is 100 — (%H + %C + %N).

The determination of the ultimate composition of ammonium tartrate by this method gave the results shown in table 10. Analytical values were taken from table 5. The accuracy in this example is within 1 percent of the true values. The method is capable of good precision and accuracy because it employs true constants, and the assumptions reside in the analyses (completeness of oxidation, accuracy of C and N analyses, correct designation of oxidation end products). This method is usable on most natural samples even though several trace organic elements are disregarded. For example, the difference between the determined (assuming complete oxidation and accurate C and N analyses) and theoretical hydrogen content for protein containing 1 percent sulfur (values from Krogh and Berg, 1931) is only 0.08 percent absolute (i.e., 7.00 percent vs. 7.08 percent H). A nomogram can be prepared from equation 3 to facilitate the calculation of hydrogen, according to the direction in Levens (1948).

CONCLUDING REMARKS

Of the three simple, direct conversions of an Oxygen Consumed datum that have been described, the estimation of organic energy (gcal. = mg. O × 3.4) is probably the most valid and widely applicable. Three transformations employing more than one analytical datum have also been considered. Among these, the determination of proximate composition from the experimental Oxygen Equivalent and organic nitrogen estimate appears to be the most useful. These six derivations fulfill the objectives of this study but do not fully explain the uses of oxidative analysis. The reader is referred to Kuhn and L'Orsa (1931) for a discussion of partial oxidations in the determination of functional groups, and to Williams (1937) for a method of determining the molecular weight of pure organic isolates.

Part VI. LIMNOLOGICAL EXAMPLES OF QUANTATIVE OXIDATION

During the development of the foregoing oxidative techniques, different samples from various sources were investigated to aid in improving the methods as well as to demonstrate their application. Some of these analyses have already been presented, either as specific examples of methods or in support of assumptions. Other analyses have been concerned with a continuing program of regional limnology in the Sierra Nevada area of California.[3] The following three examples represent such pilot studies; their immediate ob-

[3] U.S. Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife, project: California-Nevada Sport Fishery Investigations with headquarters in Reno, Nev.

jective is associated with the present research problem but their data are contributory to the continuing limnological program.

TOTAL ORGANIC CONTENT OF ALPINE LAKES

Measurement of the standing crop of net plankton is perhaps the most widely used approach in describing the productive status of waters. However, when a vertical plankton tow from a depth of 270 feet yields no detectable catch, the task of making within-lake or between-lakes comparisons becomes rather formidable. This condition of alpine oligotrophy has been observed by Reimers et al. (1955). It is suggestive of the problems encountered in the measurement and interpretation of alpine productivity which originally prompted this study. The analyses of these waters, therefore, afford a prime example of the application of quantitative oxidation in limnological investigation.

Selected descriptive features and analytical data for four lakes in the Convict Creek drainage of the Sierra Nevada are given in table 11. These lakes lie within a circle about 2 miles in diameter and border within 1.5 miles of the principal crestline. Their oligotrophic nature is induced primarily by extrinsic factors related to altitude. Net plankton was not found in two lakes (Dorothy, Cloverleaf) during the initial survey in 1950. More extensive limnological descriptions of these lakes appear in the published survey report (Reimers et al., 1955).

TABLE 11.—*Limnological features and results of oxidative analyses of four lakes in the Sierra Nevada, Calif.*

A. Descriptive features

[Data from Reimers et al. (1955)]

Lake	Elevation (feet)	Area (acres)	Depth (feet)			Milligrams per liter	
			Maximum	Mean	Secchi	Total solids	Hardness
Cloverleaf	10,310	11.6	25	12	bottom	20	9.0
Bighorn	10,675	6.5	35	16	bottom	47	30.0
Dorothy	10,340	152	290	136	67	19	11.0
Edith	10,100	18.0	110	59	46	23	10.0

B. Sampling and analytical data

[Lakes sampled during August 1958]

Lake and location [1] and depth (feet)	Temperature (°F.)	Number of analyses	Milligrams per liter		Gram calories per liter
			Oxygen Consumed	Organic matter	
Cloverleaf:					
NW.–C–3	44	4	1.4	1.22	
NE.–C–3	45	4	1.6	1.40	
SW.–D–3	48	4	1.8	1.58	
SE.–C–3	49	4	2.1	1.84	
Mean				1.5	7.3
Bighorn:					
D–3	58	2	1.2	1.05	
D–15	54	3	1.3	1.14	
D–30	42	2	1.3	1.14	
Mean				1.1	5.4
Dorothy:					
D–15	53	5	2.1	1.84	
D–35	50	5	1.7	1.49	
D–100	39	5	1.9	1.73	
Mean				1.7	8.1
Edith: C/D–3	53	5	1.8	1.58	7.7

[1] Code letters C and D refer to sampling positions under the center of the lake surface and over the deepest point of the basin, respectively. The four arms of Cloverleaf Lake are listed by compass directions (NW., etc.).

Samples of unfiltered water were analyzed before the presently described procedures were adopted, but the only important departure from the micro method was a shorter reaction time (1.5 to 2 hours). Because of this, the oxidations were less complete and somewhat less precise than those described in part IV. Oxygen Consumed values in table 11 are unadjusted, but the derived values for organic matter and calories have been adjusted for 80-percent complete oxidation. All O.C.'s. are averages of replicate determinations and are significant to within ±8 percent where three or more analyses are combined. Absolute accuracy does not directly concern the comparison here, since all samples were analyzed similarly. Raw water includes both particulate and dissolved substances, but as the data of Birge and Juday (1934) have indicated, the dissolved fraction is strongly predominant.

The average organic matter and caloric values for each lake are not true means (weighted according to the volume at each stratum). These data also give no indication of temporal fluctuations, but the report of Birge and Juday (1927) indicates that such changes are small. The following conclusions should be viewed in the light of the above restrictions.

1. General conclusions. Total organic content of these alpine lakes apparently does not exceed 2 mg. or 10 gcal./L. Even if the assumed correction for incomplete oxidation is in error, it is unlikely that the maximum organic content of any sample is more than 3 mg./L., placing these lakes below the organically poorest ones studied by Birge and Juday (1934).

2. Within-lake comparisons. Cloverleaf Lake is extremely irregular and each of its four arms yields a different analytical quantity which correlates directly with temperature. Vertical differences in organic distribution are implied in Lake Dorothy by an intermediate minimum occurring within the three strata sampled. Significant vertical differences are not apparent in Bighorn Lake, despite a rather strong thermal gradient.

3. Between-lakes comparisons. Depth, as a characteristic of oligotrophy, does not seem to limit organic accumulation (compared with shallowness) for lakes in the same locality, as indicated by the "high" oxidation values of Lake Dorothy. On the other hand, greater depth may induce the accumulation of organic matter in alpine lakes so far as it affects the lake volume relative to rate of change of watermass. Total mineral content, indicated by total dissolved solids, apparently is not related to the organic status of solute-poor lakes. Although Bighorn Lake is highest in total dissolved solids and lowest in organic content, an inverse relationship between these factors should not be assumed. The nature of inorganic ions may be influential inasmuch as this lake has a predominant sulfate anion, while the other lakes are bicarbonate.

QUANTITATIVE PERIPHYTON ANALYSIS

The community of microorganisms which occurs on the surfaces of submerged objects, commonly termed periphyton, has been the subject of various studies (Cooke, 1956). Its accumulation on a given surface area of clean glass slides during the course of a predetermined time interval has been used as an index for comparing the productivity of Sierra Nevada lakes (Nielson, 1953). This quantitative measurement of periphyton growth is accomplished by simple gravimetry. Material removed from dried slides is weighed, ashed, and reweighed to estimate organic matter as ignition loss. One procedural disadvantage is the tedious task of removing periphyton from the slides. A second disadvantage, especially in studies on

alpine lakes, is that the amount of matter formed on the slides is frequently too small to be weighed accurately. Oxidative analysis seemed to be a likely way of obviating these disadvantages and was therefore tested by a direct comparison to the gravimetric analysis of periphyton.

Accordingly, one slide (1 × 2 inches) for oxidative analysis was included with each normal gravimetric series of four slides (1 × 3 inches) on sets made in Convict Lake, Calif., in November 1958. Two sets were made: One over the deepest point in the lake consisted of 13 series of slides spaced at 10-foot intervals from 5 feet below the surface to a depth of 125 feet, and a second set of 3 series was placed in shoal water 30 feet deep. The sets were initiated at the onset of fall overturn and were retrieved after 25 days. The four large slides in each series were processed gravimetrically,[4] and the fifth was analyzed oxidatively by the micro method.

The slides were preserved in a dry state and were oxidized by introduction directly into the reaction mixture. It was necessary to reduce the volume of 0.05 *N* dichromate to 2.0 ml. for slides from the deepest water because of their low organic complement. Analytical results were converted to organic weights and to gram calories per square decimeter of slide surface area, assuming the oxidation to be 85 percent complete. Data from both gravimetric and oxidative analyses are plotted in figure 5 with a depth-temperature curve for the lake which was measured during the periphyton growth period. Additional limnological data for Convict Lake are given by Reimers et al. (1955) and the reader is referred to Nielson (1953) for a description of periphyton apparatus and procedure for gravimetric analysis.

[4] These determinations were made by H. D. Kennedy, Convict Creek Experiment Station, Bishop, Calif. Mr. Kennedy also tentatively identified most of the periphyton organisms, which are almost exclusively algal (54 genera of algae observed, 30 of them diatoms).

A curve has been roughly fitted to the oxidative values in figure 5, and horizontal scales for both organic weight and energy are given. Gravimetric data are shown only for the deep-water set. While oxidative data can be assigned to both scales, energy units are more appropriate. The two methods of estimating periphyton quantity show good general agreement, despite the much smaller surface area (one-sixth) analyzed by oxidation. The values for each depth stratum should not necessarily coincide because one is a direct estimate of mass and the other is more nearly an estimate of energy. The difference between the two estimates in the shallower strata (above 30 feet) may represent a departure from the fixed relation of mass to energy that is assumed here (1 mg. = 5 gcal.).

The time required for the oxidative analyses was probably less than half that needed for the gravimetric analyses. Since values obtained by the latter method are not necessarily more accurate estimates of organic mass, it is concluded that the oxidative measurement of periphyton is equal, if not superior to gravimetry. It has the added advantages of sensitivity and ease of analysis, and the potential benefits of elemental (C and N) estimates.

Interpretation of the periphyton data in figure 5, apart from the methodological comparison, suggests some interesting limnological relationships. This study does not attempt to relate periphyton production to the entire lake biota, or to define rigidly its trophic status. It is assumed here that the periphyton of Convict Lake is primarily an autotrophic population (see footnote 4) quite similar in structure to nannoplankton. This is the basis for the following observations:

1. The deep-water curve indicates a logarithmic decrease in periphyton abundance with depth, analogous to a

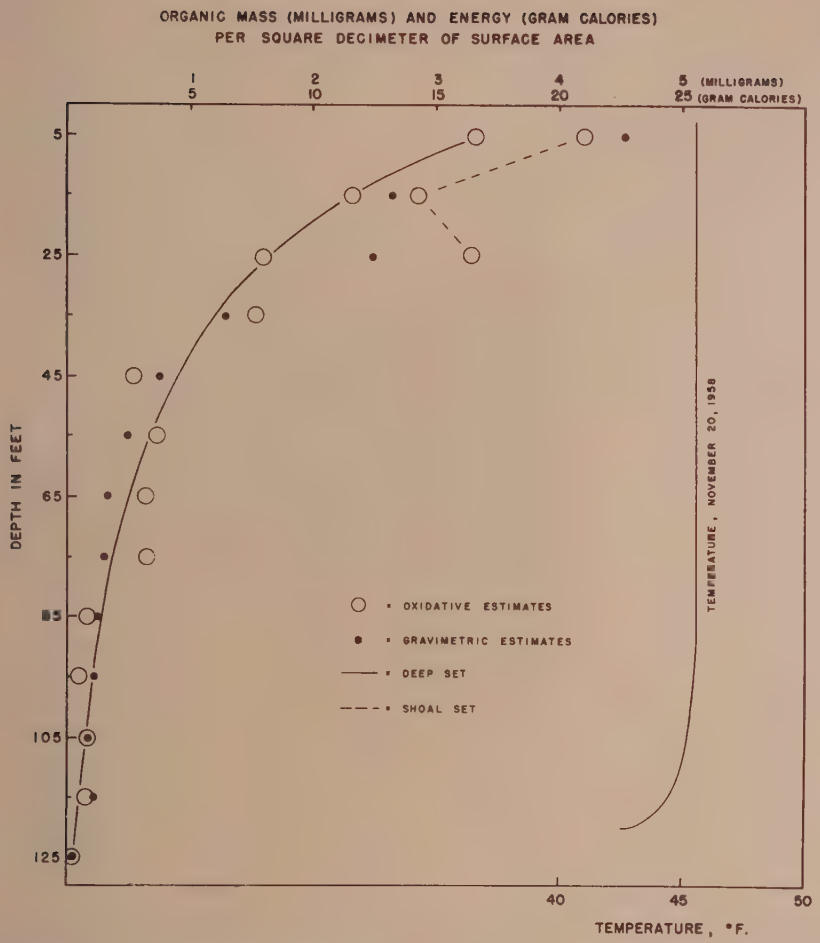

FIGURE 5.—Periphyton growth in Convict Lake, Calif., during a 25-day period (November 4–29, 1958) as determined by oxidative and gravimetric methods.

light penetration curve. Since the sampling is coincident with fall overturn, the effects of temperature and nutrient distribution are minimized. Therefore, the growth of periphyton at all depths seems dependent upon radiation intensity, and independent of vertical changes in population composition.

2. An increase in periphyton quantity is noted in the lowermost series of the shoal set. That this phenomenon is real is supported by other similar observations. This "bottom reinforcement" is probably not caused by nutrient diffusion from the sediments, but since light is evidently limiting, it is more likely attributable to a reflective increase in radiation intensity. A complex pattern of autotrophic production in the vertical dimension is suggested for the shallower waters of deep, clear lakes (or throughout shallow lakes) under conditions of light dependency, especially where bottom materials are reflective. The primary production of such a lake, induced from one or a few static vertical series of measurements, may carry a significant assumptive error.

3. A consideration of the two types of curves is indicative of the difficulty to

be expected in comparing periphyton production (rates) of lakes differing in depth and area. On the other hand, quantitative periphyton study can be a means of investigating the influence of spatial distribution and lake morphometry on a producing population.

PLANKTON AND NEUSTON COMPOSITION

Two samples of plankton were taken from Crowley Lake, California for the purpose of determining their nutrient value. One of them is a relatively pure sample of the blue-green alga *Aphanizomenon* and the other is a similar sample of the cladoceran *Daphnia*. A third sample, representing a mixed population of microorganisms, is neuston material from a pond surface near Cortland, N. Y. All of these substrates are of limnological importance insofar as they contribute to autochthonous abioseston and dissolved organic matter upon disintegration or decomposition. The neuston material is probably of little significance in lakes but its composition apparently has not been investigated. These samples have aided in the development of the methods previously described and their Oxygen Equivalent values were shown in tables 3 and 6. The following determinations are more complete and independent of those given earlier. They are presented as a summarial demonstration of the nature and amount of information obtainable from the "complete" dichromate oxidation technique.

Analyses follow the procedures in part IV (semimicro method of oxidation with carbon and nitrogen determinations), and the interpretations of part V. The results are given in table 12. Oxygen Consumed values are lower than those previously noted because the samples had gained a few percent more moisture during storage. Oxygen Equivalents are adjusted for 5-percent incomplete oxidation, and carbon data for 3-percent incomplete recovery. The proximate composition of *Daphnia* has been calculated with and without an allowance for the distribution of nitrogen between chitin and proteinaceous materials. Observations on the derived data are summarized below.

1. The three substrates are similar in general organic respects; their O.E.'s. are close to the "average" for limnological samples.

2. The elemental percentages occur within the general range given for plankton and seston by Birge and Juday (1922), Brandt and Raben (1920), and Krogh and Berg (1931). Carbon contents are suspected to be slightly lower than their true values, perhaps due to incomplete recovery apart from incomplete oxidation.[5] Neuston nitrogen is definitely a low percentage for biological materials and may reflect a composition difference for organisms existing at the water surface ecotone.

3. Proximate composition varies more among the substrates than ultimate composition, and is therefore more distinguishing. Neuston is low in crude protein and high in crude carbohydrate and lipid, which is related to the observation above. *Daphnia* is highest in crude protein and lowest in crude carbohydrate, as expected, but is lower in lipid than other reported zooplankton analyses (see table 8).

The actual application of data such as these will depend on the nature of the study undertaken, but they are expected to be of value throughout the limnological field. The salient and concluding issue here is that a great deal of information is available from a few relatively simple analyses based on quantitative oxidation. All of the fore-

[5] A semi-independent comparison can be made by summing the assumed carbon contents of the proximate groups. Carbon percentages calculated in this manner are *Aphanizomenon* 49 percent, *Daphnia* 47 percent, and Neuston 51 percent.

TABLE 12.—*A chemical characterization of three samples of aquatic organisms by oxidative analysis*

Substrate	Analytical data (air-dry weight basis) [1]						Oxygen Equivalent	Derived data (moisture-and ash-free basis) [2]								
	Sample data		Oxygen Consumed (mg. per mg. sample)	Percentage found				Ultimate and proximate percentages							Energy, kilocalories per gram	
	Number analyzed	Weight range (mg.)		Carbon	Nitrogen	Ash-free dry weight		Carbon	Nitrogen	Hydrogen	Oxygen	Crude protein	Lipid	Crude carbohydrate	Sample	Carbon
Aphanizomenon	4	22.5–26.9	1.191	40.4	6.83	90	1.41	46.6	7.6	8.1	37.6	47.6	4.6	47.8	4.80	10.3
Daphnia	5	19.0–31.2	0.988	32.0	7.13	71	1.46	46.8	10.1	9.0	34.1	63.0	4.6	32.4	4.97	10.6
Neuston	4	20.5–21.9	1.094	39.1	4.34	80	1.43	50.3	5.4	6.7	38.6	[3] 54.1 33.6	[3] 6.8 8.7	[3] 39.1 57.7	4.86	9.7

[1] Analytical results are unadjusted means.
[2] Oxygen Equivalents adjusted for 5-percent incomplete oxidation, percentage carbon for 3-percent incomplete carbon recovery. Caloric values do not include energy of nitrogen oxidation, which can be calculated (see part V).
[3] Adjusted for 20 percent chitin, a value assumed from Geng (1925).

going data for any sample may be determined from a single "complete" oxidation, assuming the organic content of the sample is known. The entire analytical procedure requires one weighing, one volumetric dilution, 3 accurate pipettings, and 3 titrations for which only 2 standardized reagents are required.

Part VII. SUMMARY

A scheme is presented for the characterization of natural organic matter which is based on its reaction with acid dichromate. Conditions of strong acidity and prolonged heating enable dichromate to oxidize nearly all organic compounds effectively, returning substituent elements to normal inorganic states. This reaction provides a basis for three analyses. The principal analysis is a measurement of the amount of reacting oxygen (quantitative oxidation) and is described in two related procedures: the semimicro method, applicable mainly to weighable particulate samples; and the micro method, designed especially for minute amounts of organic matter in deciliter volumes of water. The other two analyses concern the diffusion separation and estimation of organic carbon and nitrogen as reaction end products (CO_2 and NH_3). All analyses are accomplished titrimetrically, and emphasis is placed on procedural simplicity.

These analyses apply broadly to limnological samples, based on an evaluation of organic oxidation states. Natural organic matter is considered a mixture of compounds in which carbon is reduced from the dioxide by bonding which excludes some oxygen from the molecules. The extent of this reduction is distinctive for different compounds and chemically similar classes. The amount of oxygen required for reoxidation (Oxygen Consumed) is related to the energy, mass and composition of sample organic matter. These relationships are summarized in the following diagram (fig. 6). The limnological application of this analytical scheme is demonstrated with samples of plankton, periphyton and alpine lake water.

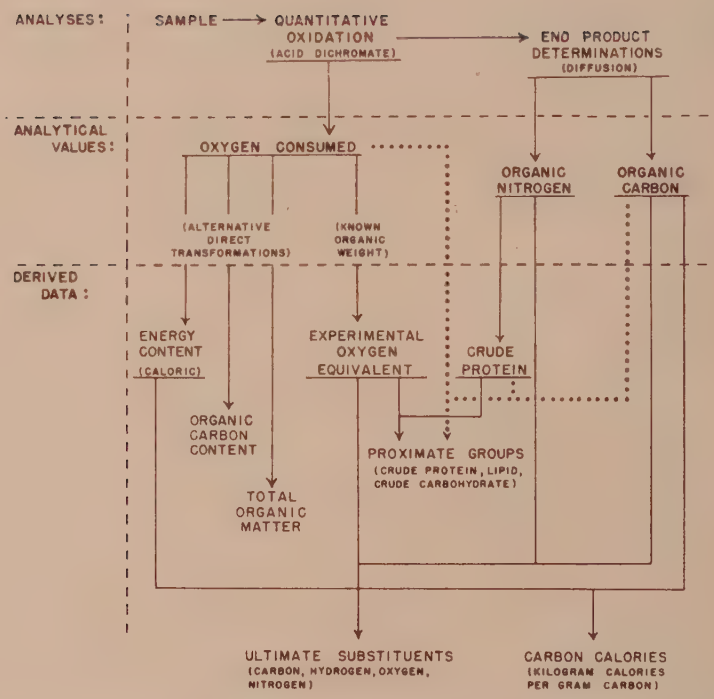

FIGURE 6.—Flow diagram of the salient features of organic analysis by quantitative oxidation.

BIBLIOGRAPHY

ÅBERG, B., and W. RODHE.
1942. Über die Milieufaktoren in einigen südschwedischen Seen. Symbolae Botanicae Upsaliensis, 5: 1–256.

ALEYEV, B.
1934. Secretion of organic substances by algae into the surrounding medium. Mikrobiologiya, 3(4): 508. (English summary).

ALLEN, F., and J. LUCK.
1929. The oxidation of dixanthydryl urea by means of the dichromate reaction. Journal of Biological Chemistry, 82(3): 693–701.

ALLISON, L.
1935. Organic soil carbon by reduction of chromic acid. Soil Science, 40: 311–320.
1960. Wet combustion apparatus and procedure for organic and inorganic carbon in soil. Soil Science Society of America, Proceedings, 24(1): 36–40.

AMERICAN PUBLIC HEALTH ASSOCIATION.
1955. Standard methods for the examination of water, sewage, and industrial wastes. 10th ed., New York. 522 p.

AMES, J., and E. GAITHER.
1914. Determination of carbon in soils and soil extracts. Journal of Industrial and Engineering Chemistry, 6: 561–564.

ARCHER, E.
1954. A semi-micro wet combustion method for the determination of carbon. Analyst, 79(934): 30–33.

ARK, H.
1928. An investigation of the oxidation of certain aliphatic and aromatic carbon compounds by means of the dichromate reaction. Chemical Society, Journal, 5: 46–47.

BAKER, N., H. FEINBERG, and R. HILL.
1954. Analytical procedures using a combined combustion-diffusion vessel. Simple wet-combustion method suitable for routine carbon-14 analyses. Analytical Chemistry, 26(9): 1504–1506.

BATTLEY, E.
1957. Carbon determination with alkaline persulfate in the Warburg manometer. Journal of Biological Chemistry, 226: 237–240.

BERG, K., and I. PETERSEN.
1956. Studies on the humic, acid Lake Gribsø. Folia Limnologica Scandinavia, No. 8. 265 p.

BIRGE, E., and C. JUDAY.
1922. The plankton. I. Its quantity and chemical composition. Wisconsin geological and Natural History Survey, Bulletin 64. 222 p.
1927. The organic content of the water of small lakes. American Philosophical Society, Proceedings, 66: 357–372.
1934. Particulate and dissolved organic matter in inland lakes. Ecological Monographs, 4(4): 440–474.

BLOCK, R.
1959. The approximate amino acid composition of wild and hatchery trout *(Salvelinus fontinalis)* and some of their principal foods *(Gammarus* and *Hexagenia bilineata)*. Boyce Thompson Institute, Contributions, 20(1): 103–106.

BRANDT, K., and E. RABEN.
1920. Zur Kenntnis der chemischen Zusammensetzung des Planktons und einigen Bodenorganismen. Wissenschaftliche Meeresuntersuchungen, Abschnitt, Kiel, N.F., 19: 175–210.

BRAUN, R.
1952. Limnologische Untersuchungen an einigen Seen im Amazonasgebeit. Schweizerisch Zeitschrift für Hydrologie, 14: 1–128.

BRAUNS, F.
1952. The chemistry of lignin. Academic Press, New York. 808 p.

BURLEW, J. (editor).
1953. Algal culture from laboratory to the pilot plant. Carnegie Institute, Publication 600. 357 p.

BUYDENS, R., and R. LEDENT.
1957. Determination of the oxidizability of waters. Chemical Abstracts, 52: 9490f, 1958. (Bulletin Centre Belge Étude et Document Eaux, 4: 238–241.).

CHEN, S., and K. LAUER.
1957. Carbon determination in biological material with a persulfate oxidation method. Analytical Chemistry, 29: 1225.

CHRISTENSEN, B., R. WILLIAMS, and A. KING.
1937. Organic oxidation equivalent analysis. III. General method using dichromate. American Chemical Society, Journal, 59 (2): 293–296.
CLARK, H., and A. MAZUR.
1941. The lipids of the diatoms. Journal of Biological Chemistry, 141(1): 283–289.
CLARK, N., and C. OGG.
1942. A wet combustion method for determining total carbon in soils. Soil Science, 53: 27–35.
COLLINS, G., J. DRUMMOND, T. HILDITCH, and E. GUNTHER.
1934. Observations on the fatty constituents of marine plankton: II. General character of the plankton oils. Journal of Experimental Biology, 11(2): 198–202.
CONWAY, E.
1958. Microdiffusion analysis and volumetric error. 4th ed. Crosby Lockwood and Son, London. 465 p.
COOKE, W.
1956. Colonization of artificial bare areas by microorganisms. Botanical Review, 22(9): 613–638.
DOBRZHANSKAYA, M.
1956. Seasonal and daily fluctuations in the content of dissolved organic matter in the Black Sea. Doklady Akademiia Nauk S.S.S.R., 111(2): 462–465.
DOMOGALLA, B., C. JUDAY, and W. PETERSON.
1925. The forms of nitrogen found in certain lake waters. Journal of Biological Chemistry, 63(2): 269–285.
DRACHEV, S., and O. MITYAGINA.
1957. The effect of ultraviolet irradiation on the organic substances in water. Chemical Abstracts, 51: 17467g, 1957. (Vestnik Akademiia Med. Nauk S.S.S.R., 12(3): 61–63.)
DUURSMA, E.
1960. Dissolved organic carbon, nitrogen and prosphorus in the sea. Vrije Universitat Amsterdam. 147 p.
ELLIS, M., B. WESTFALL, and M. ELLIS.
1948. Determination of water quality. U.S. Fish and Wildlife Service, Research Report 9, 122 p.
ELSTER, H.
1955. Beiträge zur Limnochemie der Hochschwarzwaldseen. I. Archiv für Hydrobiologie Supplement, 22: 1–64.
1959. Beiträge zur Limnochemie der Hochschwarzwaldseen. III. Archiv für Hydrobiologie, 24(3): 361–384.
EL WAKEEL, S., and J. RILEY.
1957. The determination of organic carbon in the marine muds. Conseil international pour l'exploration de la mer. Journal du Conseil, 22(2): 180–183.
FOGG, G.
1953. The metabolism of algae. John Wiley & Sons, N.Y., 149 p.
1959. Dissolved organic matter in oceans and lakes. New Biology, 29: 31–48.
FORSBLAD, I.
1955. Eine Mikromethode zur Bestimmung von organisch gebundenem Kohlenstoff in Seewasser. Mikrochimica Acta, 1: 173–186.
FRIEDEMANN, T., and A. KENDALL.
1929. The determination of carbon and carbon dioxide. Journal of Biological Chemistry, 82(1): 45–55.
GENG, H.
1925. Der Futterwert der natürlichen Fischnahrung. Zeitschrift für Fischerei, 23: 137–165.
GILLBRICHT, M.
1957. Ein Verfahren zum oxydativen Nachweis von organischen Subtsanz im Seewasser. Helgoländer Wissenschaftliche Meeresuntersuchungen, 6(1): 76–83.
GORYUNOVA, S.
1948. Dissolved organic matter in open water. Chemical Abstracts, 42(21): 8034d, 1948. (Mikrobiologiya, 17: 271–281.)
1950. Mucous substances of *Oscillatoria*. Chemical Abstracts, 44: 6488a, 1950. (Izvestia Akademiia Nauk S.S.S.R., Ser. Biologiya, 29–42.)
1952. Characterization of dissolved organic substances in water of Glubokoje Lake. Trudy Institut Mikrobiologiya Akademiia Nauk S.S.S.R., 2: 166–179.
1954a. Characterization of dissolved organic substances in water of Beloje Lake. Trudy Institut Mikrobiologiya Akademiia Nauk S.S.S.R., 3: 185–193.
1954b. Secretions occurring during the growth of the diatom alga *Synedra* sp. Trudy Institut Mikrobiologiya Akademiia Nauk S.S.S.R., 3: 194–200.
GRAHAM, E.
1948. Determination of soil organic matter by means of a photoelectric colorimeter. Soil Science, 65: 181–184.
HAUROWITZ, F.
1955. Biochemistry. John Wiley & Sons, New York. 485 p.
HILL, R., and C. WHITTINGHAM.
1958. Photosynthesis. 2d ed., John Wiley & Sons, New York. 175 p.
HODGMAN, C. (editor in chief).
1959. Handbook of chemistry and physics. 41st ed., Chemical Rubber Publishing Co., Cleveland. 3472 p.

HSIAO, S.
1949. A limnological study of Erh Hai. 2. Biological features. Journal of Animal Ecology, 18: 89–99.
HUTCHINSON, G.
1957. A treatise on limnology. I. Geography, physics and chemistry. John Wiley & Sons, New York. 1015 p.
IVLEV, V.
1934. Eine Mikromethode zur Bestimmung des Kaloriengehalts von Nahrstoffen. Biochemische Zeitschrift, 275: 49–55.
JOHNSON, M.
1949. A rapid micromethod for estimation of non-volatile organic matter. Journal of Biological Chemistry, 181: 707.
JUDAY, C., and E. BIRGE.
1932. Dissolved oxygen and oxygen consumed in the lake waters of northeastern Wisconsin. Wisconsin Academy of Science, Arts and Letters, Transactions 27: 415–486.
KATZ, J., S. ABRAHAM, and N. BAKER.
1954. Analytical procedures using a combined combustion-diffusion vessel. Improved method for combustion of organic compounds in aqueous solution. Analytical Chemistry, 26(9): 1503–1504.
KAY, H.
1954. Eine Mikromethode zur chemischen Bestimmung des organischen Substanz im Meerwasser. Kieler Meeresforschungen, 10: 26–53.
KETCHUM, B., and A. REDFIELD.
1949. Some physical and chemical characteristics of algae grown in mass culture. Journal of Cellular and Comparative Physiology, 33(3): 281–299.
KHARASCH, M.
1929. Heats of combustion of organic compounds. Journal of Research of the Bureau of Standards, 2(2): 359–430.
KLEEREKOPER, H.
1953. The mineralization of plankton. Fisheries Research Board of Canada, Journal, 10(5): 283–291.
KLEEREKOPER, H., and F. GRENIER.
1952. The bottom sediments of Lake Lauzon, Montcalm County, Province of Quebec. I. Chemical and physical investigations. Canadian Journal of Zoology, 30(4): 219–242.
KOLTHOFF, I., and E. SANDELL.
1952. Textbook of quantitative inorganic analysis. 3d ed., Macmillan Co., New York. 759 p.
KROGH, A.
1930. Eine mikromethode für die organische Verbrennungsanalyse, besonders von gelösten Substanzen. Biochemische Zeitschrift, 221: 247–263.
KROGH, A., and A. KEYS.
1934. Methods for the determination of dissolved organic carbon and nitrogen in sea water. Biological Bulletin, 67(1): 132–144.
KROGH, A., and E. LANGE.
1932. Quantitative Untersuchungen über Plankton, Kolloide und gelöste Substanzen in dem Furesee. International Review of Hydrobiology, 26: 20–53.
KROGH, A., and K. BERG.
1931. Über die chemische Zusammensetzung des Phytoplanktons aus dem Frederiksborg-Schlosssee und Bedeutung für die Maxima der Cladoceren. International Review of Hydrobiology, 25: 204–219.
KRYLOVA, L.
1953. Micromethod for the determination of organic carbon in fresh water. Chemical Abstracts, 48: 10964b, 1954. (Gidrokhimicheskie Materialy Akademiia Nauk S.S. S.R., 20: 63–67.).
KUHN, R., and F. L'ORSA.
1931. Analyse organischer Verbindungen durch Oxydation mit Chromsaüre. Zeitschrift für Angewandte Chemie, 44(41): 847–853.
LAPUCCI, L.
1958. The potassium bichromate method for the determination of the chemical oxygen demand (C.O.D.) of water and sewage. Chemical Abstracts, 53: 17380f, 1959. (Nuovi Annales Igiene y Microbiologia, 9: 139–150.).
LAWTON, G.
1955. An investigation of the chemical oxygen demand determination. Wisconsin Academy of Science, Arts and Letters, Transactions, 44: 45–56.
LEVENS, A.
1948. Nomography. John Wiley and Sons, New York. 176 p.
LOPEZ-BENITO, M.
1955. On the determination of organic matter in seawater by means of potassium permanganate. Biological Abstracts, 31: 33734, 1957. (Invest. Pesq., 1: 67–72.).
LU, K., J. DAWSON, and M. ALEXANDER.
1959. A microchemical method for detecting antifungal substances. Archiv für Mikrobiologie, 33: 182–185.
MACFADYEN, A.
1948. The meaning of productivity in biological systems. Journal of Animal Ecology, 17: 75–80.
MASON, W.
1890. Examination of potable water. Nims & Knight, Troy, N.Y. 48 p.

MATIDA, Y., and H. SAKURAI.
1953. Chemical analysis of organic adhesive detritus on river gravels. Japanese Society Sci. Fisheries, Bulletin, 19: 31–33. (English summary).

MATSUE, Y.
1950. Phytoplankton and its oxidizability by permanganate. Japanese Society of Sci. Fisheries, Bulletin, 15: 813–817. (English summary).

MAUCHA, R.
1932. Hydrochemische Methoden in der Limnologie. Die Binnengewässer, 12. Schweizerische Verlagsbuchhandlung, Stuttgart. 173 p.

MEDALIA, A.
1951. Test for traces or organic matter in water. Analytical Chemistry, 23(9): 1318–1320.

MEISSNER, B.
1958. Der Kaliumpermanganat verbrauch in der Wasseranalyse. Wasserwirtschaft-Wassertechnik, 8(1): 14–20.

MILNER, H.
1948. The fatty acids of *Chlorella*. Journal of Biological Chemistry, 176(2): 813–817.

MOHLMAN, F., and G. EDWARDS.
1931. Determination of carbon in sewage and industrial wastes. Industrial and Engineering Chemistry, Analytical Edition, 3(2): 119–123.

MOORE, W., R. KRONER, and C. RUCHHOFT.
1949. Dichromate reflux method for determination of oxygen consumed. Analytical Chemistry, 21: 953–957.

MOORE, W., and W. WALKER.
1956. Determination of low chemical oxygen demands of surface waters by dichromate oxidation. Analytical Chemistry, 28: 164–167.

NIELSON, R.
1953. Apparatus and methods for the collection of attachment materials in lakes. Progressive Fish-Culturist, 15(2): 87–89.

NIKOLAEVA, E.
1953. On the dichromate method of determining the oxidation of organic substances in fresh water. Gidrokhimicheskie Materialy Akademiia Nauk S.S.S.R., 20: 68–78.

OHLE, W.
1940. Chemische Eigenart der smålandischen Seen. International Association of Theoretical and Applied Limnology, Proceedings, 9: 145–159.

PEECH, M., L. ALEXANDER, L. DEAN, and J. REED.
1947. Methods of soil analysis for soil-fertility investigations. U.S. Department of Agriculture, Circular 757. 25 p.

PETERSON, W., E. FRED, and B. DOMOGALLA.
1925. The occurrence of amino acids and other nitrogen compounds in lake water. Journal of Biological Chemistry, 63(2): 287–295.

PHILLIPS, A., H. PODOLIAK, D. LIVINGSTON, R. DUMAS, and R. THOESEN.
1959. Cortland hatchery report No. 28. New York State Conservation Department, Albany. 83 p.

PONOMAREVA, V., and A. ETTINGER.
1953. The nature of organic substances dissolved in the water of the Neva River. Doklady Akademiia Nauk S.S.S.R., 88: 105–108.

PTITSYN, B.
1956. Oxidation potential of dichromate. Chemical Abstracts, 51: 14383i, 1957. (Zhurnal Obshchei Khimii, 26: 3233–3239.).

REIMERS, N.
1958. Conditions of existence, growth, and longevity of brook trout in a small high-altitude lake of the eastern Sierra Nevada. California Fish and Game, 44(4): 319–333.

REIMERS, N., J. MACIOLEK, and E. PISTER.
1955. Limnological study of the lakes in Convict Creek Basin, Mono County, California. U.S. Fish and Wildlife Service, Fishery Bulletin 103: 437–503.

RICHMAN, S.
1958. The transformation of energy of *Daphnia pulex*. Ecological Monographs, 28: 273–291.

ROGERS, R., and W. ROGERS.
1846. On a new process for obtaining formic acid, and on the preparation of aldehyde and acetic acid by the use of the bichromate of potassa. American Journal of Science, Series 2, 2(4): 18–24.
1848. New method of determining the carbon in native and artificial graphites. American Journal of Science, Series 2, 5 (15): 352–359.

SCHOLLENBERGER, C.
1927. A rapid approximate method for determining soil organic matter. Soil Science, 24(1): 65–68.
1945. Determination of soil organic matter. Soil Science, 59: 53–56.

ŠESTÁK, Z.
1958. Methods of determining organic carbon in water and their use in hydrobiology. Acta Universitatis Carolinae, Biologica Vol., 1958 (3): 269–281. (English summary).

SHAPIRO, J.
1957. Chemical and biological studies on the yellow organic acids of lake water. Limnology and Oceanography, 2(3): 161–179.
1961. Freezing-out, a safe technique for concentration of dilute solutions. Science, 133 (3470): 2063–4.
SHAW, K.
1959. Determination of organic carbon in soil and plant material. Journal of Soil Science, 10(2): 316–326.
SKOPINTSEV, B.
1948. Organic matter in some waters in Podmoskov'e. Chemical Abstracts, 43: 789i, 1949. (Doklady Akademiia Nauk S.S.S.R., 61: 293–296.).
1949a. Determination of organic carbon in fresh water. Chemical Abstracts, 44: 3181e, 1950. (Zhur. Anal. Khimicheskie, 4: 192–198.).
1949b. Consumption of oxygen by stable organic substances in natural waters. Chemical Abstracts, 45: 9776h, 1951. (Gidrokhimicheskie Materialy Akademiia Nauk S.S.S.R., 16: 61–71.).
1949c. Determination of organic matter in native waters. Evaluation and comparison. Chemical Abstracts, 47: 7702h, 1953. (Trudy Biogeokhimicheskie Lab. Akademiia Nauk S.S.S.R., 9: 67–126.).
1950. The relation between the oxidation forms of oxygen, organic carbon and total organic matter content of natural waters. Chemical Abstracts, 47: 5589b, 1953. (Gidrokhimicheskie Materialy Akademiia Nauk S.S.S.R., 18: 57–58.).
SKOPINTSEV, B., and L. MIKHAILOVSKAYA.
1948. A qualitative characterization of the organic substances in natural waters. Chemical Abstracts, 45: 5846h, 1951. (Gidrokhimicheskie Materialy Akademiia Nauk S.S.S.R., 14: 108–114.).
SLATER, J.
1954. The quantitative evaluation of dissolved organic matter in natural waters. American Microscopical Society, Transactions, 73(4): 416–423.
SMITH, S.
1952. Studies in the development of rainbow trout (*Salmo irideus*). II. The metabolism of carbohydrates and fats. Journal of Experimental Biology, 29(4): 650–666.
SPECTOR, W. (editor).
1956. Handbook of biological data. W. B. Saunders Co., Philadelphia. 584 p.
SPOEHR, H., and H. MILNER.
1949. The chemical composition of *Chlorella;* effect of environmental conditions. Plant Physiology, 24: 120–149.
STRECHER, P. (editor).
1960. Merck index of chemicals and drugs. 7th ed., Merck & Co., Rahway, N.J. 1641 p.
STRICKLAND, J.
1960. Measuring the production of marine phytoplankton. Fisheries Research Board of Canada, Bulletin 122. 172 p.
STRICKLAND, J., and T. PARSONS.
1960. A manual of sea water analysis. Fisheries Research Board of Canada, Bulletin 125. 185 p.
STUNDL, K.
1951. Microchemistry in water analyses. Mikrochimie Vereinigung Mikrochimica Acta 36/37: 927–932.
THORNTON, W.
1917. The relation of oxygen to the heat of combustion of organic compounds. Philosophical Magazine, 33: 196–203.
TIURIN, I.
1931. A new modification of the volumetric method of determining soil organic matter by means of chromic acid.. Pedology (Pochvovedenie), 26(5–6): 46–47. (English summary).
URBANOWICZÓWNA, I.
1947. Determination of organic matter in water. Chemical Abstracts, 42: 2039b, 1948. (Roczniki Chemji, 21: 59–63.).
VALLENTYNE, J.
1957. The molecular nature of organic matter in lakes and oceans, with lesser reference to sewage and terrestrial soils. Fisheries Research Board of Canada, Journal, 14(1): 33–82.
In press. Geochemistry of the carbohydrates. *In* Bregor, Organic geochemistry. Pergammon Press, N.Y.
VAN DE VELDE, A.
1936. Oxidation with potassium permanganate. Chemical Abstracts, 30: 8055_8, 1936. (Natuurwetenschappelijk Tijdschrift, 18: 191–198.).
VAN SLYKE, D., and J. FOLCH.
1940. Manometric carbon determination. Journal of Biological Chemistry, 136: 509–541.
VINBERG, G.
1954. Quantitative data on the plankton biomass of lakes of White Russia, SSR. Chemical Abstracts, 51: 14909a, 1957. (Uchenye Zapiski Belorussky Gosudarst Univ. im V.I. Lenina, Ser. Biologiya, No. 17: 20–37.).
VINBERG, G., V. IVLEV, T. PLATOVA, and L. ROSSOLIMO.
1934. Methodik der Bestimmung des organischen Stoffes und ein Versuch der kalorieschen Schätzung der Nahrungsvor-

räte eines Beckens. Arbeiten Limnologische Sta. Kossino, 18: 38–39. (German summary).

VINOGRADOVA, Z.
1957. The biochemical composition of the Black Sea plankton. Doklady: Biological Sciences Section, 116 (1–6): 895–897. (AIBS translation, Doklady Akademiia Nauk S.S.S.R., Vol. 116.).

VOTINTSEV, K.
1953. Organic matter in the foam of Lake Baikal. Doklady Akademiia Nauk S.S.S.R., 92(2): 425–427.
1955. Vertical distribution and seasonal variation of organic matter in the water of Lake Baikal. Doklady Akademiia Nauk S.S.S.R., 101(2): 359–363.

WAKSMAN, S., and I. HUTCHINGS.
1935. Chemical nature of organic matter in different soil types. Soil Science, 40: 347–363.

WALKLEY, A.
1935. An examination of methods for determining organic carbon and nitrogen in soils. Journal of Agricultural Science, 25: 598–609.
1947. Critical examination of a rapid method for determining organic carbon in soils—effect of variations in digestion conditions and inorganic soil constituents. Soil Science, 63: 183–197.

WALKLEY, A., and I. BLACK.
1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Science, 37: 29–38.

WASHBURN, E. (editor).
1929. Heats of combustion of some organic compounds. International Critical Tables, 5: 162–169.

WELCH, P.
1952. Limnology. 2d ed., McGraw-Hill Book Co., New York. 538 p.

WEREŠČAGIN, G., N. ANIČKOVA, and T. FORSCH.
1932. Methoden der hydrochemischen Analyse in der limnologischen Praxis. Archiv für Hydrobiologie, 23: 167–230.

WEST, E.
1956. Textbook of biophysical chemistry. Macmillan Co., New York. 399 p.

WILLIAMS, R.
1937. Organic oxidation equivalent analysis. I. Theory and applications. American Chemical Society, Journal, 59(2): 288–290.

APPENDIX—PROXIMATE COMPONENTS IN LIMNOLOGICAL SAMPLES

The proximate components (carbohydrates, fats, and proteins) can be considered as groups of chemically related compounds. These component groups have been used in characterizing limnological organic matter by Birge and Juday, Krogh and coworkers, and many others. Carbohydrates, fats, and proteins, as described and discussed in general biochemical texts, are quite distinct entities, but analytical partitioning of total sample organic matter into three proximate groups necessarily places some less closely related compounds together. It is improper, then, to consider carbohydrates only as hexoses or hexose polymers, fats only as fatty acids or triglycerides, and proteins only as amino acids or their condensates. To emphasize this difference, the analytical proximate groups are referred to here as crude protein, lipid, and crude carbohydrate.

The most common proximate analysis determines lipid by organic solvent extraction, crude protein by conversion of the measured organic nitrogen, and crude carbohydrate as the amount of residual matter. Obviously, organic nitrogen is present in compounds other than proteins or their constituent molecules, and solvent extraction removes some nonfatty materials. Residual "carbohydrate" then contains all compounds not otherwise accounted for, as well as the analytical errors of the other two determinations. It is felt that these proximate groups would have greater significance and utility in limnological studies if they were more closely defined in terms of the most important compounds actually present. Furthermore, it is necessary for the analytical scheme presented here to evaluate the more important oxidative characteristics of the proximate groups and interpret them as representative values. In doing this, it is desirable to view the proximate groups as they are determined by the more common analytical techniques so that the results of oxidative analysis will be compatible with data of other research.

The characterization of proximate components has been accomplished by arranging the most abundant limnological compounds (or representatives) and their characterizing data into groups and subgroups (appendix tables A–1 through A–5). Empirical formulas and energy contents (kcal./g. substrate) were taken mainly from Hodgman (1959) and Washburn (1929). From these data, the percentages of carbon and nitrogen, the Oxygen Equivalents (text, p. 7), and the other energy relations were calculated. The most useful energy value in oxidative analysis is kilocalories per gram of oxygen consumed (or gcal./mg. O.C.), which Vinberg et al. (1934) have referred to as the "oxycalorie coefficient." Calorie values for nitrogenous compounds do not include energy of nitrogen oxidation (see text, p. 39). This compilation and its interpretation are based on the works of Fogg (1953), Haurowitz (1955), Vallentyne (1961), and especially Vallentyne (1957), with lesser references noted in context. A great many compounds which would be included in this classification chemically, have not been

demonstrated as quantitatively important in limnological material. On the other hand there are doubtless several prominent natural compounds which deserve inclusion but remain to be described chemically. The listed compounds are assumed to be sufficient in number and type to permit a satisfactory introductory characterization of proximate groups. This characterization is a tentative personal interpretation of existing information and may be modified by better quantitative molecular information as it becomes available.

Crude carbohydrate

A classification of carbohydrate materials is given in appendix table A–1. All of these compounds are susceptible to rapid oxidation by dichromate and, lignoids excepted, are characterized by low Oxygen Equivalents, carbon contents, and calorific values per unit compound weight, and by high oxycalorie coefficients. "True" carbohydrates appear in the upper half of the table and are considered the most abundant carbohydrate forms in cellular material such as net plankton, macrophytes, nannoplankton and periphyton. Sample material of this type is assumed to contain crude carbohydrate represented by an O.E. of 1.2, an oxycalorie coefficient of 3.5, and a carbon content of 45 percent.

Lignoids and some of the low molecular weight, high oxygen content compounds (especially acids) are expected to occur analytically as carbohydrates because of their insolubilities in organic solvents. Lignin and humic-type acids are classed together as lignoids because of similar properties (cyclic structure, methoxyl groups, high O.E. and carbon content). Abioseston and sediments may contain a large amount of lignoids (Kleerekoper, 1953). Kleerekoper and Grenier (1952) estimated that up to one-half of the organic material at the surface of bottom sediments is lignin. Lignoids are also prominent components of dissolved organic matter especially in humic waters. Although Shapiro (1957) has observed organic solvent solubility for humolimnic isolates, the data of Birge and Juday (1927, 1934) indicate ether insolubility for dissolved humic materials. This difference may be due to different sample pretreatment. It is concluded here that the crude carbohydrate of sedimented and dissolved humic matter differs from that of cellular substance and has the representative values: carbon, 50 percent; O.E., 1.4.

APPENDIX TABLE A–1.—*Crude carbohydrates: representative compounds and some of their characteristics important in oxidative analysis*

Classification	Molecular formula	Percentage carbon	Oxygen Equivalent	Kilocalories per gram		
				Substrate	Substrate carbon	Oxygen Consumed
Hexoses and hexosans:						
Glucose	$C_6H_{12}O_6$	40.0	1.06	3.74	9.4	3.51
Starch, cellulose	$(C_6H_{10}O_5)n$	44.4	1.18	4.18	9.4	3.53
Hexosamines:						
Chitin [1]	$C_{30}H_{50}O_{19}N_4$	46.8	1.25			
Pentosans:						
Xylan	$(C_5H_8O_4)n$	45.4	1.21	4.26	9.4	3.51
Polyuronides and pectins:						
Pectic acid	$(C_6H_8O_6)n$	40.9	0.91			
Related compounds [2]:						
Lignin [3]	$C_{9.0}H_{11.4}O_{3.5}$	63.6	1.89	6.28	9.9	3.32
Humic and crenic acids	(see table 7)	49–55	1.48–1.55			
Pyruvic acid	$C_3H_4O_3$	40.9	0.91	3.16	7.7	3.49
Malic acid	$C_4H_6O_5$	35.8	0.72	2.39	6.7	3.34

[1] Composition from Strecher (1960).

[2] Examples of various compounds which may occur in this group.

[3] Data from Brauns (1952).

Lipid

Various strongly reduced, high-carbon compounds with high Oxygen Equivalents are grouped in appendix table A–2 as lipids. They are generally soluble in organic solvents, and slowly oxidized because of their immiscibility with water. Fats and fatty acids predominate in cellular material. The hypothetical triglyceride shown is based on the most abundant algal fatty acids listed by Fogg (1953). In addition to fats, extracted algal lipid may contain up to 40 percent pigments (Burlew, 1953); of these, chlorophylls are present in largest amounts (Hill and Whittingham, 1958). Free fatty acids observed in lake water (Goryunova, 1952, 1954a) were attributed to secretions from diatoms and other algae (Goryunova, 1954b). Fatty acids, pigments and their degredation products, and waxes are known from seston and sediments (Vallentyne, 1957).

The four subgroups in the upper portion of appendix table A–2 represent the most reduced states of these compounds. As decomposition or degredation proceeds, especially with fatty acids and porphyrins (pyrrole compounds), carbon contents and O.E. values decrease. Organic solvent extraction can be expected to remove some nonlipid material from a sample, and this occurrence is noted as the final group in the table. In contrast to the lipids, these compounds are of low carbon content and O.E., and more properly belong to the crude carbohydrate group.

Except in some living cells (such as diatoms or zooplankters) the total sample lipid does not exceed about 10 percent. This fact makes lipid the least abundant of the proximate groups. Nevertheless, it has a strong influence on the sample O.E. and carbon content by virtue of its highly reduced state. The characteristic values assumed here for limnological lipid are carbon 70 percent, O.E. 2.7, oxycalorie coefficient 3.3.

Crude protein

The nitrogen-containing materials include the most important biological compounds and frequently comprise the largest fraction of sample organic matter. However, the group appears to be poorly understood in proximate analysis of limnological material and is therefore given special consideration.

Amino acids and the larger molecules into which they condense are considered "true" proteins. Elemental analyses of purified proteins indicates that they are very nearly 16 percent nitrogen, and it is from such "text" values that the conversion factor for estimating crude protein is obtained. Individual amino

APPENDIX TABLE A–2.—*Lipids: representative compounds and some of their characteristics important in oxidative analysis*

[illegible]	Molecular formula	Percentage carbon	Oxygen Equivalent	Kilocalories per gram		
				Substrate	Substrate carbon	Oxygen Consumed
Fats, oils and fatty acids [1]:						
Palmitic acid	$C_{16}H_{32}O_2$	75.0	2.86	9.36	12.5	3.26
Palmitolic acid	$C_{16}H_{30}O_2$	75.2	2.79	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Triglyceride (of the above)	$C_{51}H_{94}O_6$	76.9	2.85	[illegible]	[illegible]	[illegible]
Pyrrole compounds:						
Chlorophyll pheophytin	$C_{55}H_{74}O_5N_4$	75.9	2.50	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[1] Molecules assumed to be representative from the data given in Fogg (1953).

[2] Determined values for organic solvent extracts (ether and benzene-methanol) of algae (Fogg, 1953) and sediments (Vallentyne, 1957).

APPENDIX TABLE A–3.—*Characteristics of some free amino acids from aquatic plant and animal protein hydrolyzates*

Amino acid	Molecular formula	Percentages		Oxygen equivalent	Kilocalories per gram		Order of abundance [1]		
		Carbon	Nitrogen		Amino acid	Oxygen consumed	Algae	Invertebrates	Brook trout
Leucine and isoleucine	$C_6H_{13}O_2N$	55.0	10.7	1.829	5.92	3.24	1	1	1
Glutamic acid	$C_5H_9O_4N$	40.8	9.5	0.979	3.15	3.22	3	2	2
Aspartic acid	$C_4H_7O_4N$	36.1	10.5	0.721	2.31	3.20	5	3	5
Lysine	$C_6H_{14}O_2N_2$	49.2	19.1	1.530	5.09	3.32	6	5	6
Alanine	$C_3H_7O_2N$	40.4	15.7	1.077	3.48	3.23	8	7	4
Valine	$C_5H_{11}O_2N$	51.2	12.0	1.640	5.29	3.23	2	9	8
Arginine	$C_6H_{14}O_2N_4$	41.4	32.1	0.989	3.49	3.45	7	4	9
Proline	$C_5H_9O_2N$	52.1	12.2	1.529	5.09	3.33	4	8	10
Glycine	$C_2H_5O_2N$	32.0	18.7	0.639	2.08	3.25	11	14	3

[1] Based on data given by Fogg (1953) and Block (1959).

acids, as shown in appendix table A–3, actually contain quite dissimilar nitrogen percentages. However, the average contents of nitrogen for aggregates of free amino acids or for proteins are much less variable. Other characterizing values also can be represented by averages. Thus, amino acid carbon content is about 50 percent, Oxygen Equivalent is about 1.5, and energy content is about 5 kilocalories per gram. Oxcalorie coefficients, averaging nearly 3.3, vary the least. The above values increase slightly when calculated for combined acids because one water molecule is lost for each peptide bond formed. The calculated O.E.'s and energy contents of several proteins are given in appendix table A–4. On the whole, they are quite similar (O.E. 1.49 to 1.60; kcal./g. 4.92 to 5.29) but a distinct difference is evident between the algal and animal proteins, the former having a higher average O.E. and caloric content.

In some biological materials, the assumption that total organic N is reckonable as protein may be very nearly correct (Haurowitz, 1955, noted that less than 2 percent of human blood nitrogen is nonprotein). Limnological samples may be strikingly different, and this assumption can cause a rather misleading conception of crude protein. Nitrogenous compounds have been listed in the two previous groups (carbohydrate chitin and lipid pyrroles). Others are given in the lower part of appendix table A–5. These nonprotein nitrogenous compounds have different characteristic values (especially percentage N and O.E.) and, if present in a sample in substantial amounts, may cause a significant error in the crude carbohydrate estimate. The calculation of crude protein (organic N $\times$ 6.25) mathematically assigns at least some of any nitrogen-containing material to this group. Geng (1925) has shown that invertebrates may contain up to 20 percent chitin (properly, a carbohydrate). The

APPENDIX TABLE A–4.—*Oxygen Equivalents and caloric values of some aquatic plant and animal proteins*

[Based on compositions given by Block (1959) and Fogg (1953)]

Protein source	Oxygen equivalent [1]	Kilocalories per gram of protein [2]
Algae:		
Anabaena (Cyanophyta)	1.58	5.21
Chlorella (Chlorophyta)	1.56	5.15
Navicula (Bacillariophyta)	1.59	5.26
Tribonema (Chrysophyta)	1.60	5.29
Aquatic invertebrates:		
Gammarus (Crustacea)	1.49	4.92
Ephemeroptera (Insecta)	1.53	5.06
"Mixed insects" [3]	1.52	5.02
Trout (*Salvelinus*):		
Wild	1.49	4.92
Hatchery	1.49	4.92

[1] Calculated by adjusting all amino acid percentages reported for each source to 100 and summing their individual weighted Oxygen Equivalents (combined acid O.E. = free acid O.E. $\times$ mol. wt./mol. wt. — 18).

[2] Calculated from Oxygen Equivalents and oxycalorie coefficients (kcal./g. = O.E. $\times$ 3.3). Corresponding calorimeter values would be about 0.88 kilocalories per gram higher.

[3] Chiefly Plecoptera.

APPENDIX TABLE A–5.—*Crude proteins: representative compounds and some of their characteristics important in oxidative analysis*

Classification	Molecular formula	Percentages		Oxygen equivalent	Kilocalories per gram		
		Carbon	Nitrogen		Substrate	Substrate carbon	Oxygen consumed
Proteins [1]:							
1	$C_{4.32}H_{7.00}O_{1.50}N_{1.14}$	52	16	1.44	4.8	9.2	3.3
2	$C_{4.45}H_{7.00}O_{1.49}N_{1.11}$	53.5	15.6	1.48			
Amino acids	(see table A-3)	32–55	10–32	0.6–1.8	2–8		3.3
Related compounds [2]:							
Hexosamines:							
Chitin	$C_{30}H_{50}O_{19}N_4$	46.8	7.4	1.24	4.3	9.3	3.5
Pyrrole compounds:							
Pheophytin	$C_{55}H_{74}O_5N_4$	75.9	6.4	2.50			
Nucleic acid derivatives:							
Adenine	$C_5H_5N_5$	44.5	51.9	.59			
Uracil	$C_4H_4O_2N_2$	42.9	25.0	.71			
Excretory products:							
Uric acid	$C_5H_4O_3N_4$	35.7	33.3	.29	.91	2.5	3.2

[1] Data for protein No. 1 from Krogh and Berg (1931) and for protein No. 2 from Brandt and Raben (1920). See appendix table A–4 for other values.

[2] Examples of nitrogen-containing compounds placed in this group by analytical definition.

aforementioned calculation partitions chitin between the two proximate groups; an example of this error is demonstrated in text table 12. On the other hand, if strongly nitrogenous excretory products are present (such as urea or uric acid) the conversion error places an excess quantity of matter into the crude protein group and diminishes the crude carbohydrate proportion which is calculated by difference. The latter effect cannot be demonstrated by existing data, but must surely occur to some extent on occasion. Because crude protein by analytical definition is organic N $\times$ 6.25, these errors cannot be resolved easily until more is known about the molecular nature of limnological organic-nitrogen compounds.

Cellular material can be expected to contain a predominance of protein nitrogen. The studies of Domogalla et al. (1925) and Peterson et al. (1925) have indicated that most dissolved organic N is proteinaceous (amino and peptide). More recently, Vallentyne and others (see Vallentyne, 1957) have identified several amino acids from hydrolyzates of dissolved and sedimented organic materials. Other important nitrogenous compounds are poorly characterized.

The data given for proteins, amino acids, and nonprotein nitrogenous compounds in appendix table A–5 suggest the following representative values for crude protein: Carbon, 52 percent; O.E., 1.5; oxycalorie coefficient, 3.3. This O.E. is possibly too high for dissolved matter, and for particulate matter where living plant cells are not abundant. A crude protein O.E. of 1.45 or 1.4 may prove to be more appropriate in such instances.

☆ U. S. GOVERNMENT PRINTING OFFICE: 1962—643393

Created by Act of Congress in 1849, the Department of the Interior is responsible for a wide variety of programs concerned with the management, conservation, and wise development of America's natural resources. For this reason it often is described as the "Department of Natural Resources."

Through a score of bureaus and offices the Department has responsibility for the use and management of millions of acres of federally owned lands; administers mining and mineral leasing on a sizable area of additional lands; irrigates reclaimed lands in the West; manages giant hydroelectric power systems; administers grazing and forestry programs on federally owned range and commercial forest lands; protects fish and wildlife resources; provides for conservation and development of outdoor recreation opportunities on a nationwide scale; conserves hundreds of vital scenic, historic, and park areas; conducts geologic research and surveys; encourages mineral exploration and conducts mineral research; promotes mine safety; conducts saline water research; administers oil import programs; operates helium plants and the Alaska Railroad; is responsible for the welfare of many thousands of people in the Territories of the United States; and exercises trusteeship for the well-being of additional hundreds of thousands of Indians, Aleuts, and Eskimos, as well as being charged with resource management of millions of acres of Indian-owned lands.

In its assigned function as the Nation's principal natural resource agency, the Department of the Interior bears a special obligation to assure that our expendable resources are conserved, that renewable resources are managed to produce optimum yields, and that all resources contribute their full measure to the progress, prosperity, and security of America, now and in the future.